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Histopathological Observations on Experimental *Aeromonas hydrophila* Injection in *Clarias batrachus* (Linn.)

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ABSTRACT

One hundred healthy walking catfish were experimentally injected intramuscularly with 0.1 ml. of normal saline (0.85% NaCl) containing 2.56×10^8 cells of *Aeromonas hydrophila*. Histopathological changes of skin, muscle, gills and internal organs were studied.

Histopathological observations indicated that the gills and the internal organs appeared normal, whereas inflammatory responses of skeletal muscle of the early stage was detected. It was characterized by diapedesis of blood vessels in the hypodermal layer, hydropic degeneration of muscle fibres and myoseptum. Advanced stage was characterized by the appearance of dead cells of epidermis, dermis and hypodermis and formation of ulcerative lesions on the body surface.

The natural healing process was observed and characterized by the migration of epithelial cells to cover the lesion. In addition, fibrosis and granulation were observed by day 4. The lesion was completely healed within 3 weeks after the bacterial injection.

INTRODUCTION

Aeromonas hydrophila is the bacterial species distributed widely in the aquatic environment such as saline waters, estuaries and freshwater (Allen *et al.*, 1983; De Figuiredo and Plumb, 1977; Hazen *et al.*, 1978; Hepher, 1981). The density of *A. hydrophila* was higher in lotic than in lentic system (Hazen *et al.*, 1978. Hepher (1981) reported that the number of *A. hydrophila* in higher salinity water i.e. > 15 ppt was low.

The bacterial septicaemia is the most important disease in cultured catfish and is often found in farms where trash fish is used as a feed and in multiple culture system, where *A. hydrophila* was initially involved (Anonymous, 1981; Areerat *et al.*, 1982). Furthermore, Areerat and her coleagues (1982) reported that bacterial septicemia generally occurs at 2 weeks post - hatching and at 3 - 3 1/2 months of age. Lolbrera and Gacutan (1987) reported that *A. hydrophila* was isolated from the diseased fish during the ulcerative disease outbreak in Laguna de Bay during December 1985 to February 1986. Also, *A. hydrophila* is the major pathogenic bacteria associated with the outbreak of ulcerative disease in the Indo - Pacific region (Tonguthai, 1985). The other cited references also indicate that

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A. hydrophila is largely involved in the bacterial disease of tropical fish population.

The purpose of this experiment was to determine the histopathological changes and healing process of the skin, muscle, gills and internal organs of *C. batrachus* which were experimentally injected with *A. hydrophila*

MATERIALS AND METHODS

One hundred healthy walking catfish averaging 15 - 20 cm. in total body length bought from a private farm were maintained at the National Inland Fisheries Institute (NIFI) Laboratory. Twenty - five fish were acclimatized for one month in glass aquaria which contained 150 liters of underground water with fully aerated. The experimental fish were fed with commercial pellets at 4% of body weight. For maintenance of the water quality total changing of water in the aquarium was done every 2 days.

Pure culture of *A. hydrophila* from the NIFI Laboratory was sub - cultured in Brain Heart Infusion agar (BHI). The *A. hydrophila* was injected through catfish twice in order to enhance virulence before used. Bacterial density was measured by spectrophotometer at the wave length of 540 nm. and the poured plated method was used for counting the bacterial colonies. The anesthesized fish were injected intramuscularly with 0.1 ml. of the bacterial suspension in normal saline (0.85% NaCl) containing 2.56×10^8 cells of bacteria. Control fish were injected with the same amount of isotonic saline. The inoculated fish were kept in the aquaria for further observation.

Three injected and one controled fish specimens were sampled at 1, 2, 4, 6, 8, 10, 12, 24, 36 hours and 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 21, 28 and 35 days after injection. The fresh at the site of injection and also the internal organs (gill, heart, kidney, spleen, liver and intestine) were fixed immediately in 10% cold buffered formalin for at least 24 hours, and then processed routinely for histology as described by Humason (1979).

RESULTS

Gross Pathology

The injected walking catfish stayed quietly at the bottom of the aquarium during the first day after inoculation.

An oval or irregular white lesion appeared by 24 hours at the injection site. The necrotic muscle was present at the center of the lesion and it sloughed off causing an open concave lesion by 4 - 7 days. By 10 - 14 days the size of the ulcerative lesion of the injected fish decreased with a darked colour at the edge of the lesion. Around 21 - 35 days most of the experimental fish showed darkened depressed scars on the body. None of these changes was demonstrated in the control fish.

Pathological Changes

Histopathological changes caused by *A. hydrophila* injection were observed. Diapedesis of the blood vessels in the hypodermis and hydropic degeneration of muscle fibers were demonstrated at one hour after injection, especially in a small area around the injected site (Figure 1 and Figure 2). The necrotic muscle fibers were increased in numbers and followed by swollen myoseptum at 2 and 4 hours after injection (Figure 3).

At 6 hours of bacterial inoculation more sarcolysis and haemorrhage were observed. At this stage lymphocytes and macrophages started appearing in the defeated

area (Figure 4). However, haemorrhage and sarcolysis were expanded and created a bigger area of lesion at 8 - 10 hours. At the same time, a few polymorphonuclear leucocytes (PMN) or neutrophiles were encountered into the wounded area with some bacterial colonies (Figure 5). The necrosis of muscle bundles increased at 12 hours. Around 24 hours after the inoculation an advanced lesion with dead cells of epidermis, dermis and hypodermis started to slough off from the body of the injected fish (Figure 6). The lesion could be observed clearly as the whitish lesions surrounded by haemorrhage on the body of the inoculated fish. More advance ulcerative lesions were demonstrated at 36 - 48 hours after injection.

At day 3 the epidermal cells of the regenerated epidermis migrated down toward the depth of the wound to form the new epidermis to cover the open lesion (Figure 7). The dead material at the center of the ulceration was slough off by day 4 and the epithelialization was completed. At the same time, fibroblasts, melanin pigment containing cells (Figure 8) and new blood capillaries were found at the defeated area. At day 5 fibrosis was very active and the basement membrane of the epidermal layer was completely formed but dermis and hypodermis still, separated. Haemorrhage was observed within the necrosis area. Histopathological observation of the defeated area in 6 - 7 days were quite the same as those at day 5. Besides the new muscle fibers regeneration were first observed.

Small number of melanin pigment containing cells began to be encountered under the basement membrane of the epidermis at 8 - 9 days. At day 10 the damage portion of the dermis were still disconnected. More melanin pigment containing cells were observed both in the epidermis and under the basement membrane of the epidermal layer (Figure 9).

After 12 - 13 days, the separated dermis was almost joined by the fibrous connective tissues that were arranged in the parallel position to the epidermal layer. Around day 14 fibrosis was still observed under the joined epidermis. The dead cells formed due to sarcolysis decreased in number. In 21 - 35 days the regenerated muscle bundle almost replaced the fibrosis area. Active macrophages were still found with the small focal lymphocyted accumulation in the healing area.

After 4 weeks, the lesion appeared to be normal (Figure 10) under histopathological observation except that more melanin containing cells were found in the dermal layer. The scar was still observed with naked eye even at the 35 days after injection. Ten infected catfish in this experiment did not show histopathological changes of the internal organs, except from the moribund specimens that found ascites, focal necrosis and bacterial colonies in heart, liver, kidney and spleen.

DISCUSSION

The gross pathological changes of the infected walking catfish were similar to those observed in snakehead by Chinabut (1989). The lesion of infected fish began to heal around 10 - 14 days and completely healed by day 28 to 35. The healing sites were distinguished grossly as dark concave scars. This was contrasted with the report of Ventura and Grizzle (1987) who induced septicaemias in channel catfish by intramuscular inoculation of sublethal dose of *A. hydrophila* and produced a light coloured area at the site of injection.

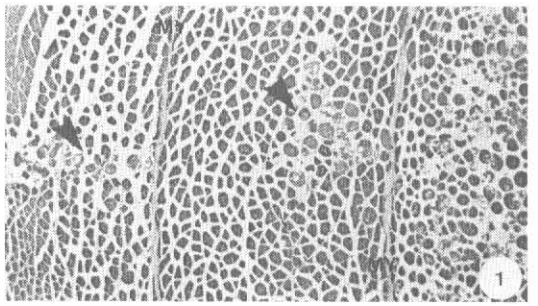

Figure 1. Inflammatory response in the muscle bundles at 1 hour after injection : necrotic muscle fibers area (arrow) ; MY = myoseptum. (Formalin; H&E; X50).

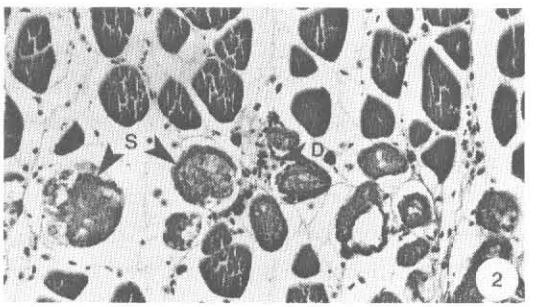

Figure 2. The enlargement of Figure 1. demonstrated diapedesis (D) among the sarcolysis (S) of muscle bundles. (Formalin; H&E; X264).

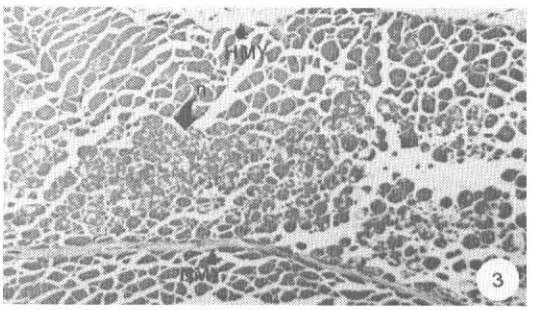

Figure 3. Necrotic muscle fibers and hydropic degeneration of myoseptum at 2 hours after injection: n = necrotic area; NMY = normal myoseptum; HMY hydropic degeneration of myoseptum. (Formalin; H&E; X50).

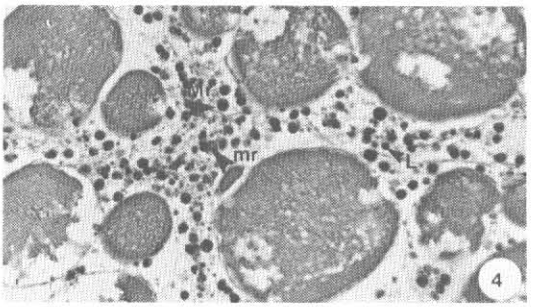

Figure 4. Inflammatory response in between the necrotic muscle at 6 hours after injection : L = lymphocyte; Mr = swollen macrophage; mr = macrophage cell; Rb = red blood cell. (Formalin; H&E; X50).

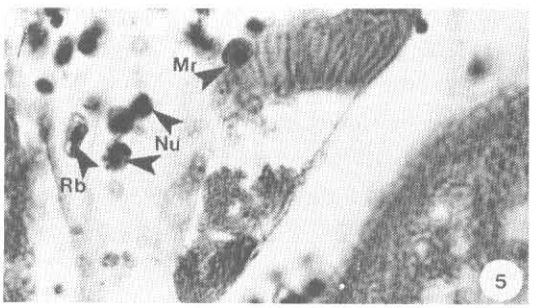

Figure 5. PMN or neutrophil in the infected area at 8 hours: Nu = neutrophil; Mr = macrophage; Rb = red blood cell. (Formalin; H&E; X1320).

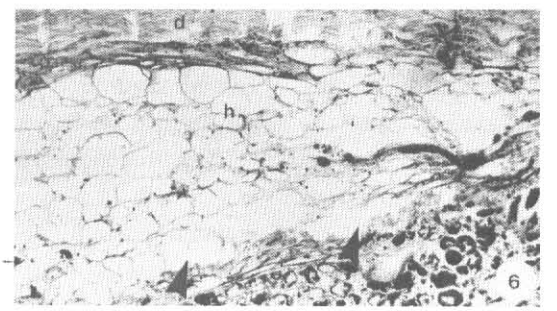

Figure 6. Sloughing off the dead tissue (arrow) from the body at 24 hours; d = dermis; h = hypodermis. (Formalin; H&E; X132).

Apparently, the peak of cell damaging processes caused by bacterial activities was within 24 - 48 hours. It was indicated by large ulcerative lesions on the flank and these may cause death of some of the infected fish (Lerssuthichawal, 1987 : Charnchit, 1985).

The polymorphonuclear leucocytes (PMN) infiltrated into the inflammatory area at 8 hours after injection and increased in numbers while the lesion progressed. Nagamura and Wakabayashi (1985) also reported that polymorphonuclear leucocyte infiltration was a primary response to *A. hydrophila* infection of Japanese eel, *Anguilla japonica*. there was no evidence of clustering of PMN around the bacterial colonies as described in infected striped snakehead (Chinabut, 1989). These may explain that the response of PMN to bacterial infection in difference fish species was dissimilar.

The defence mechanism pattern of walking catfish against *A. hydrophila* was similar to that of striped snakehead. These observations demonstrated that healthy walking catfish can tolerate to certain amount of *A. hydrophila* infection, if they stay in a good environmental condition.

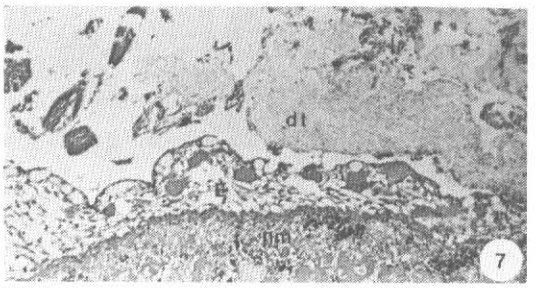

Figure 7. Epithelial cells migrated to cover the lesion at days 3; dt = dead tissue; E = epidermis; nm = necrotic muscle fiber. (Formalin; H&E; X132).

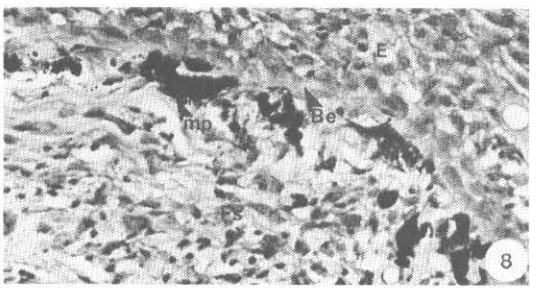

Figure 8. Fibrosis under the new epidermis at days 4; E = new epidermis; Be = basement of epidermis; mp = melanin pigment; Fs = fibrosis. (Formalin; H&E; X528).

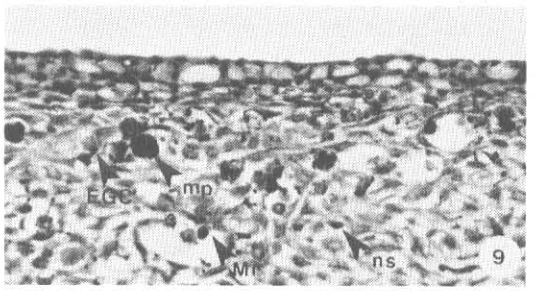

Figure 9. Melanin pigment (mp) and eosinophilic granular cells (EGC) in the epidermis at day 10: Mr = macrophage; ns = nucleus of squamous epithelial cell. (Formalin; H&E; X528).

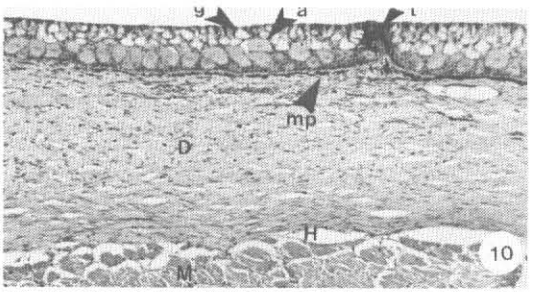

Figure 10. The skin of infected fish (10 a) at day 28 which was similar to the skin of control fish (10b) : g = goblet cell; a = alarm substance cell; t = taste buds; mp = melanin pigment; E = epidermis; D = dermis; H = hypodermis; M = muscle bundle. (Formalin; H&E; X132).

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