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A Histopathological Study of Motile Aeromonad Disease in Ayu

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In the autumn of 1978, motile aeromonad disease (*Aeromonas hydrophila*) occurred among ayu, *Plecoglossus altivelis* being cultured in ponds in Tokushima Prefecture. Symptoms of the infected fish were exophthalmia with hemorrhage and cutaneous hemorrhage in the tail and anus. Histopathological observation of exophthalmic eyes indicated extensive bacterial invasions with hemorrhage and necrosis in the conjunctiva and orbital adipose tissue, congestive choroid and separated retina. Liver showed focal hemorrhage. Spleen showed hemorrhage of pulp. Kidney showed nephrosis and hemorrhage of the hematopoietic tissue.

Introduction

Aeromonas hydrophila infects various species of freshwater fishes. Recently, this bacterial infection was commonly called as motile aeromonas septicemia (AFS 1975). As severe hemorrhage and ulceration of the body surface are visible signs of the infected fishes, this infection had been called as red pest for European eel, *Anguilla anguilla* (SCHÄPERCLAUS, 1934), red disease for Japanese eel, *A. japonica* (HOSHINA, 1962), red disease for carp, *Cyprinus carpio* (EGUSA, 1978) and red sore for largemouth bass, *Micropterus salmonides* (HUIZINGA *et al.*, 1979). *A. hydrophila* infection was also found among pond cultured ayu, *Plecoglossus altivelis* (JO *et al.*, 1980). However, the clinical and histopathological signs were different from those reported on other fishes. This report describes specific characteristics of those signs.

Materials and Methods

Five ayu (15-20 g body weight) infected with motile *A. hydrophila* were collected from ponds in Tokushima Prefecture in October of 1978. After observation of clinical signs and isolation of the bacterium from visceral organs of the fish, the head, heart, gills, liver, spleen, kidney and digestive tracts were fixed in 10% formalin solution and then sectioned with routine histological procedures. Tissue sections were stained with

Mayer's hematoxylin and eosin, Giemsa, PAS and Azan stain.

Results

Clinical and histopathological signs

General clinical signs of diseased fish were exophthalmia and cutaneous hemorrhage in the tail and anus (Fig. 1).

In the affected, exophthalmic eyes (Fig. 2), the conjunctiva was extensively invaded by the bacteria resulting in epithelial separation, necrosis, edema and hemorrhage. The cornea was erosive and necrotic although bacterial invasions were not obvious. Capillaries of the iris were engorged with blood, accompanying hemorrhage and bacterial invasions. The retina was separated without



Fig. 1. External view of an infected ayu showing exophthalmic eyes.

obvious bacterial invasions. Bacteria were disseminated into the choroid and multiplied intensively in the blood vessels which were necrotized, followed by extensive hemorrhage, edema, infiltration of neutrophils and fibrin precipitation (Fig. 3). In hemorrhagic lesions, bacteria were also found among erythrocytes and in fibrin, and phagocytosis of neutrophils against bacteria was not obvious. Connective tissue of the sclera beside the affected choroid was necrotized. The orbital adipose tissue showed extensively hemorrhage with neutrophil infiltration and bacterial dissemination (Fig. 4). The oculo-motor musculature was edematous and necrotic without obvious bacterial invasions (Fig. 5). In liver, sinusoids were congested with slightly migrated bacteria (Fig. 6). Hepatocytes lost fat and were slightly atrophic but bacterial invasions into hepatocytes were not found. In spleen, pulps were engorged with blood, followed by hemorrhage and the destruction of the tissue (Fig. 7). Sheathed tissue of sheathed arteries was hemorrhagic. Slight bacterial migration was found in the hemorrhagic pulps. In kidney, epithelia of renal tubules were necrotized without bacterial invasions. Sinusoids of the hematopoietic tissue were extensively congested and hemorrhagic with very slight bacterial migration (Fig. 8). Reticulo-endothelial cells were found to be necrotic. In heart, cardiac muscle underwent cloudy swelling with no bacterial invasion, although very slight bacterial migration was found in the blood of the cardiac lumen.

On the other hand, stomach, pyrolic caecum and intestine (Fig. 9) showed congestion, while gills

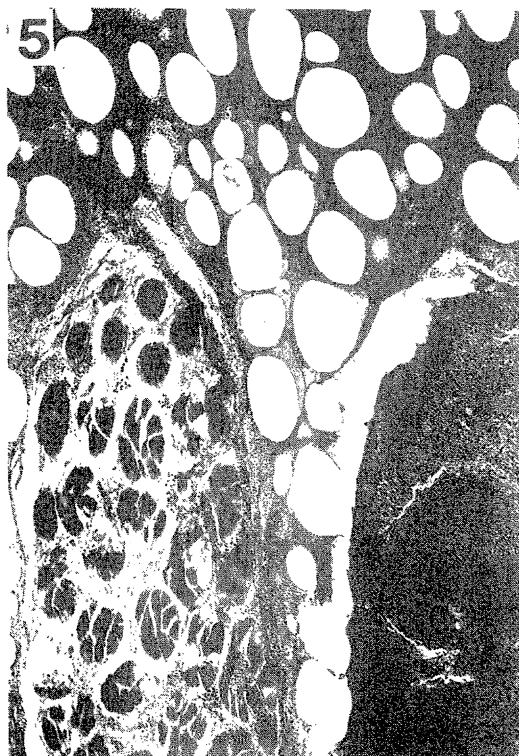
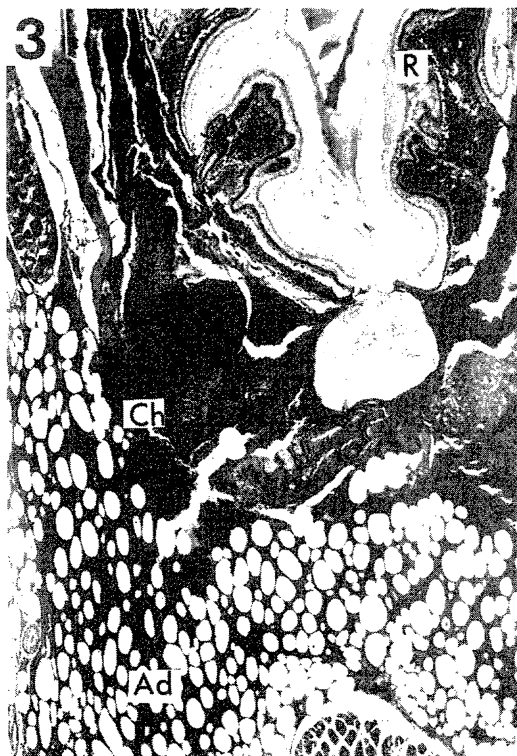
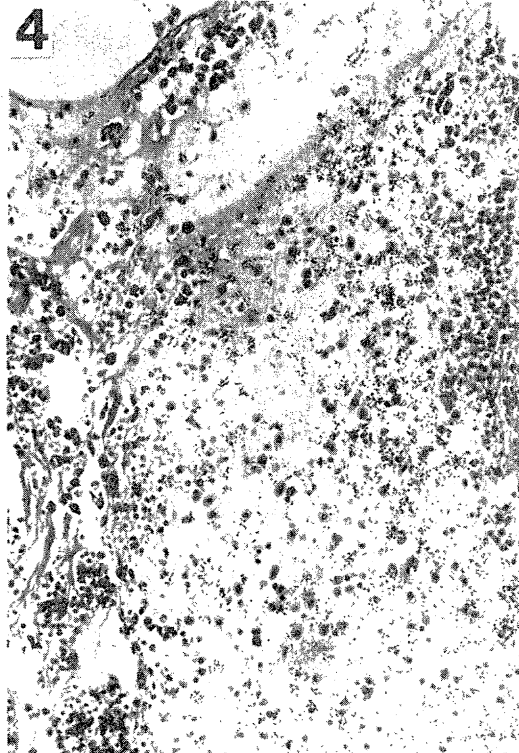
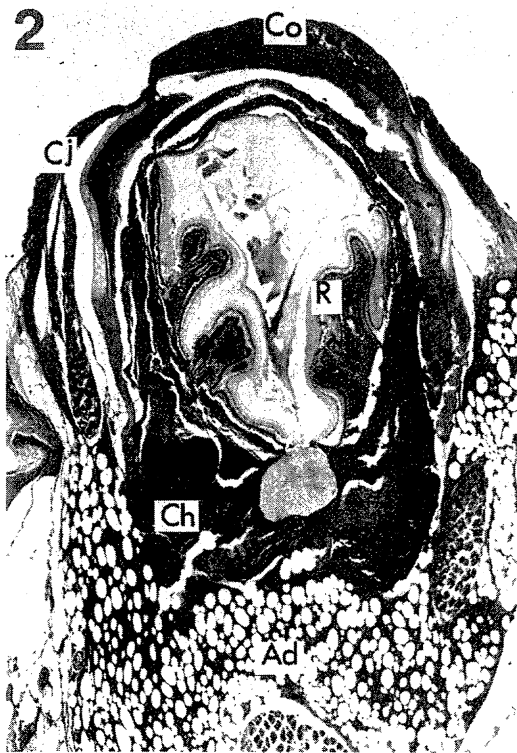
and brain showed no pathological change.

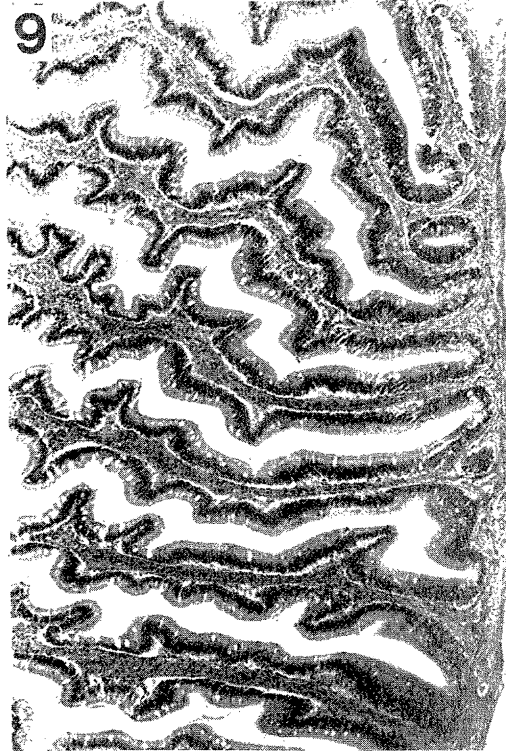
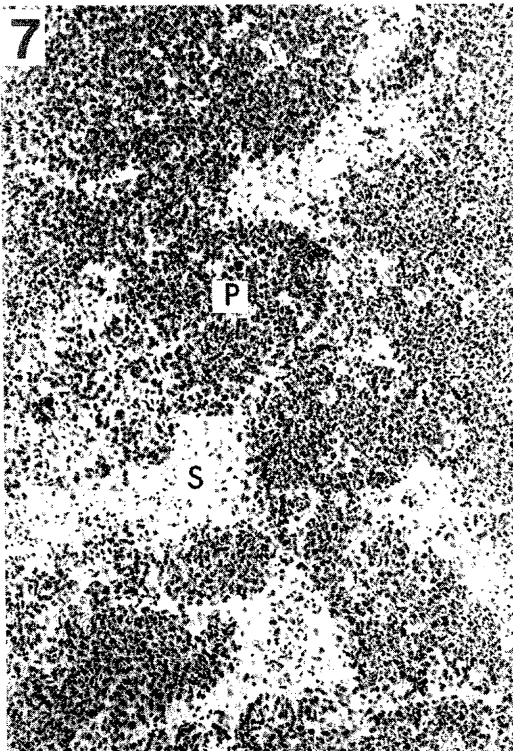
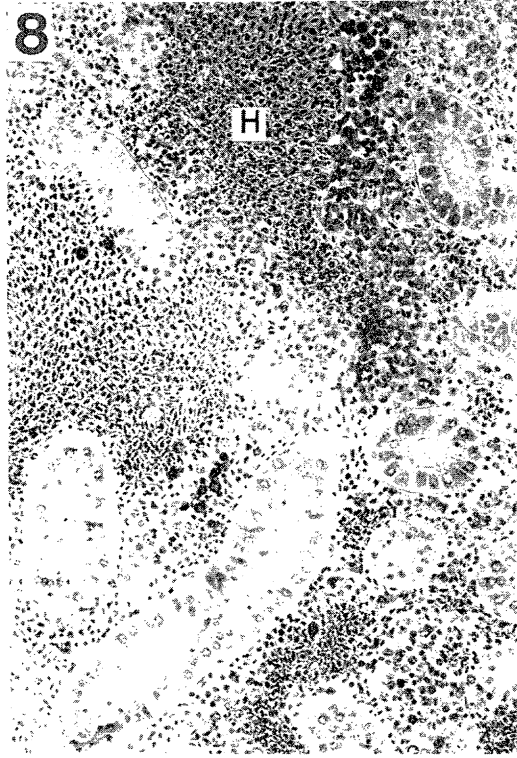
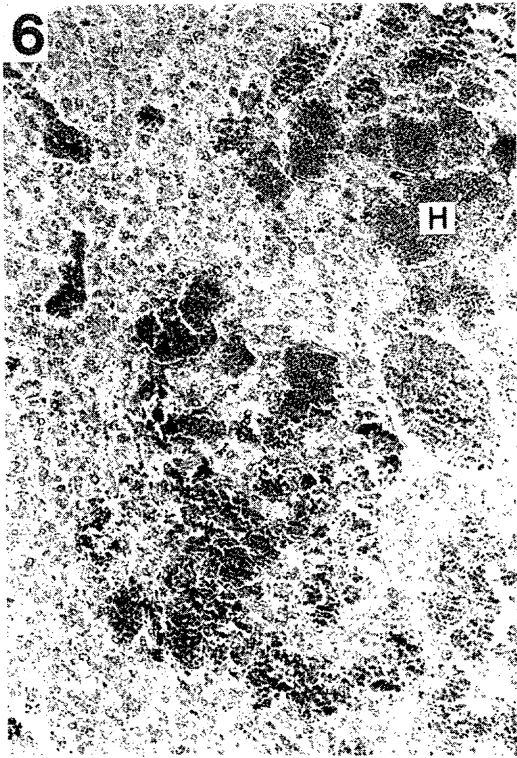
Discussion

Aeromonas hydrophila is usually recognized as an orally infectious bacterium (EGUSA, 1978), however, the intestine in case of ayu showed no obvious pathological change. In addition, the eye was noticed to be the first organ for the infection. This finding indicated that the bacteria invade primarily the tissue of eyes instead of the intestine. Circulatory disturbances and cellular damage were found to develop in the liver, spleen, kidney and heart, although the bacterial migration was very slight in those organs. This fact indicated that the bacterial multiplication was confined in the affected eyes and the above pathological changes of visceral organs were evoked by affection of toxic substances of the bacterium instead of bacterium itself. In *A. hydrophila* infection, the affection of toxic substances of the bacterium has been proposed to evoke circulatory disturbances and cellular damage in naturally infected European eel (SCHÄPERCLAUS, 1934), Japanese eel (EGUSA 1978, MIYAZAKI, 1980), largemouth bass (HUIZINGA *et al.*, 1979) and channel catfish, *Ictalurus punctatus* (WOLKE, 1975). This was experimentally confirmed *in vivo* (SHIMIZU 1969, a, b; ALLAN *et al.*, 1981; WAKABAYASHI *et al.*, 1981; KANAI *et al.*, 1984). Results on the above experiments indicated that pathogenicity of *A. hydrophila* was due to protease and hemolysin. A test *in vitro* revealed this bacterium possessing elastase (WAKABAYASHI *et al.*, 1981). These bacterial enzymes probably damage blood

Explanation of Figures

- Fig. 2.** Exophthalmic eye. Co: cornea with epithelial sloughing and degeneration. Cj: necrotic and hemorrhagic conjunctiva. R: separated retina. Ch: choroid with congestion and hemorrhage. Ad: adipose tissue with hemorrhage. H-E stain, $\times 10$.
- Fig. 3.** A detail of exophthalmic eye. R: separated retina. Ch: choroid with congestion and hemorrhage. Ad: adipose tissue with hemorrhage. H-E stain, $\times 16$.
- Fig. 4.** Infected orbital adipose tissue. Bacterial multiplication, hemorrhage, edema and fibrin precipitation were observed. Giemsa stain, $\times 200$.
- Fig. 5.** A detail of orbital adipose tissue and oculo-motor musculature showing hemorrhage and necrosis. H-E stain, $\times 50$.
- Fig. 6.** Liver showing hemorrhage (H) and congestion. H-E stain, $\times 160$.
- Fig. 7.** Spleen showing engorgement with blood in pulps (P). S: sheathed artery. H-E stain, $\times 80$.
- Fig. 8.** Kidney showing hemorrhage in the hematopoietic tissue (H). H-E stain, $\times 160$.
- Fig. 9.** Intestine showing congestion and no bacterial multiplication in the lumen. H-E stain, $\times 80$.





vessels resulting in hemorrhage because blood vessels are mainly composed of elastic and collagenous fibers (MIYAZAKI *et al.*, 1977).

A. hydrophila infection has been named as motile aeromonas septicemia. On histopathological examination, however, obvious septicemic condition could not be confirmed in ayu as reported on infected European eel, Japanese eel and largemouth bass. Septicemic condition usually accompanied systemic bacterial infection which was commonly observed in the infection of *Vibrio anguillarum* (FUNAHASHI *et al.*, 1974) and *Streptococcus iniae* (MIYAZAKI, 1981) of ayu. Thus toxemia is more suitable in case of ayu for characterization of *A. hydrophila* infection.

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