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# A Histopathological Study of *Pseudomonas fluorescens* Infection in Tilapia

Teruo MIYAZAKI*1, Saburoh S. KUBOTA*2 and Toshio MIYASHITA*3

*1*Faculty of Fisheries, Mie University, 2–80, Edobashi, Tsu, Mie, Japan*
*2*Nippon Veterinary and Zootechnical College, Kyonan-cho, Musashino, Tokyo, Japan*
*3*Osaka Prefectural Freshwater Fish Experimental Station, Kiyamotomachi, Neyagawa, Osaka, Japan*

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A histopathological study was made on *Pseudomonas fluorescens* infection which occurred in the spring of 1979 in pond-cultured tilapia, *Sarotherodon niloticus*. The infected fish showed exophthalmia, dark body coloration, spotty or nodular lesions in the liver, spleen, kidney and gills, and inflamed swim-bladder. The bacterial dissemination was systemic. The histopathological aspects were abscess formation in eyes, spleen and swim-bladder, and focal necrosis in the liver, gills and kidney of some diseased fish. The other fish showed granuloma formation in all infected lesions.

## Introduction

*Pseudomonas fluorescens* has been known as a causative bacterium of hemorrhagic septicemia in European eel, *Anguilla vulgaris* (ANDRÈ *et al.*, 1970) and cyprinid fishes (BULLOCK *et al.*, 1970, SHIOSE *et al.*, 1974). This bacterial infection was found also among cultured yellowtail, *Seriola quinqueradiata* (KUSUDA 1980). However, the histopathological information on this infection is rather scare. The present authors did a histopathological study on *P. fluorescens* infection of pond-cultured tilapia, *Sarotherodon niloticus* (MIYASHITA 1984). The present paper describes the clinical and histopathological aspects of the diseased tilapia.

## Materials and Methods

*P. fluorescens* infection occurred with heavy losses among tilapia in farm ponds in Aichi Prefecture in the spring of 1979. Six diseased tilapia (three 1-year old; 16.5–17.0 cm in body length. three 2-year old; 20.5–24.0 cm in body length) were sampled for the study from the ponds. After external and internal examination, bacterial isolation was tried with nutrient agar plates from visceral organs of diseased fish. Affected eyes, liver, spleen, kidney, digestive tracts, heart, swim-bladder and gills were fixed in 15% formalin solution or Bouin's fluid, and embedded in paraffin wax. Paraffin sections were stained with Mayer's hematoxylin and eosin (H-E), Giemsa, PAS, Aazn and Weigert's fibrin methods.

## Results

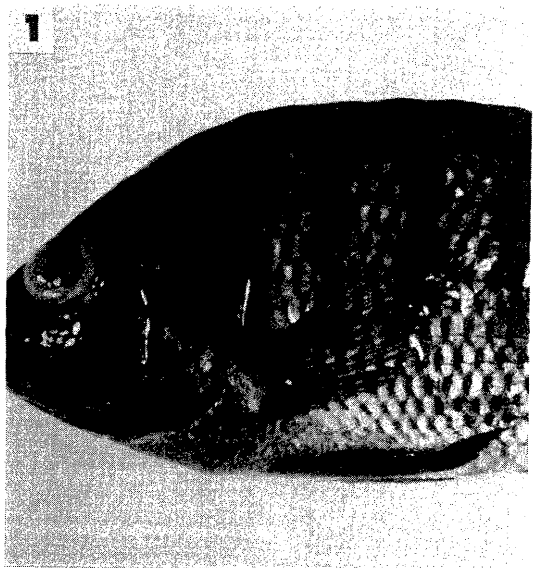
### I. External and internal signs

Diseased tilapia showed dark body coloration and exophthalmia with corneal opacity and hemorrhage (Fig. 1). Three sampled fish had the pale colored liver, swollen spleen with white nodules (Fig. 2), kidney with white spots, inflamed swim-bladder containing milky fluid in the lumen and pale colored gills with white spots. Other three fish showed white spotty lesions in the liver, spleen, kidney and gills

### II. Histopathological aspects

Histopathological changes varied with affected lesions and the progress of the infection.

**Eye**: Exophthalmic eyes of two diseased fish showed extensive accumulation of huge numbers of neutrophils with bacterial dissemination in the orbital adipose tissue. The inflammation accompanied hemorrhage and involved the optic nerve, choroid membrane, sclera, conjanctiva and oculo-motor musculature (Fig. 3). Most part of the eye ball was forced out from the orbit due to



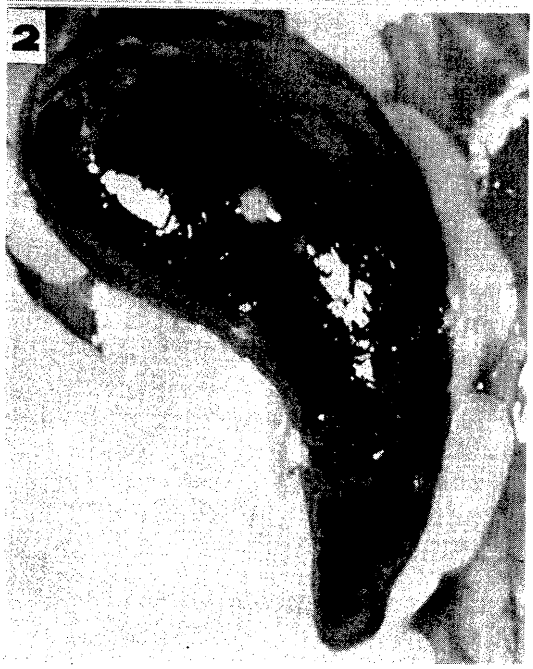


**Fig. 1.** Infected tilapia showing exophthalmia and dark body coloration.

**Fig. 2.** Dissected view of an infected fish, showing nodular lesions in the spleen.

the extensive inflammation. In the eye ball, cornea was degenerated and its epithelium was eroded. The retina was not invaded by bacteria but sloughed into the camera oculi. Bacteria multiplied mostly in amorphous exudates but were not phagocytized by neutrophils in inflamed lesions (Fig. 4).

Exophthalmic eyes of the other two fish showed the production of granulomas walling off affected tissue of the orbit and inflammatory exudates by layered epithelioid cells (Fig. 5). In granulomas, affected orbital tissue and inflammatory cells underwent coagulative necrosis. Bacteria were observed in amorphous matter and their multiplication was not obvious in granulomas. Such an inflammatory product in the orbit forced out the eye ball with degenerated cornea and sloughed retina from the orbit.

**Spleen**: Nodular lesions of spleen were histopathologically confirmed as abscesses with multiplied bacteria (Fig. 6). Each abscess was walled off by fibrin which was histochemically identified by a positive reaction to Weigert's fibrin method and red coloration with Azan stain. In the spleen with abscesses, splenic pulps were engorged with blood. Spotty lesions in the spleen were either focal necrosis or granulomas. Focal necrotic lesions were formed in pulps, accompanying with bacterial invasion and infiltration of neutrophils and macrophages. Granulomatous lesions contained coagulated cells or caseous matter (Fig. 7). In granulomas bacteria were found to disappear.

**Liver**: Histopathological examination revealed the presence of either focal necrosis or granulomas in the livers with pale coloration and spotty lesions. Focal necrotic lesions involved hepatocytes with bacterial dissemination and were accompanied by fibrin precipitation (Fig. 8). Granulomas were formed in the parenchyma and observed to contain coagulated cells or caseous matter. In granulomas, bacterial cells were hardly detected. Uninfected hepatocytes were atrophic or vacuolized.

**Kidney**: Spotty lesions in the kidney were found to be either necrotic or granulomatous lesions in the hemopoietic tissue. Necrotic lesions accompanied bacterial dissemination, infiltration of inflammatory cells and fibrin precipitation (Fig. 9). Necrotic lesions sometimes involved glomeruli and renal tubules which resulted in necrosis. Granulomas enclosed coagulated or caseous matter, in which no bacteria were observed.

**Gills**: In gills with spotty lesions, bacteria invaded connective tissue of gill filaments, causing focal necrosis, infiltration of inflammatory cells

and fibrin precipitation (Fig. 10). Some spotty lesions were packed by macrophages, in which bacterial cells were not observable. Those lesions in most cases accompaned epithelial hyperplasia.

**Swim-bladder**: Inflamed swim-bladder was diffusely invaded by bacteria and showed either neutrophil infiltration or the production of granulomas. Neutrophils extensively infiltrated in the wall, red bodies and lumen with bacterial dissemination. Feature of bacteria-phagocytosis by neutrophils was not obvious. On the other hand, granulomas extensively walled off inflammatory exudates and affected tissue which underwent coagulative necrosis (Fig. 11).

**Heart, stomach, intestine**: These organs were spared of bacterial infection.

## Discussion

*Pseudomonas fluorescens* has been known as a causative bacterium of hemorrhagic septicemia in fishes. External and internal signs of the infected fishes were similar and represented by erythemia, ulcerative skin, peritoneal hemorrhage and dropsy in European eel, *A. vulgaris* (ANDRÈ *et al.*, 1972), cyprinid fishes (BULLOCK *et al.*, 1970 SIOSE *et al.*, 197fi) and yellowtail, *S. quinqueradiata* (KUSUDA 1980). Its pathogenecity is known to be due to proteolytic enzyme (BULLOCK *et al.*, 1970). This histopathology has been studied only in European eel, in which the disease was reported to be characterized by necrosis of the hematopoietic tissue with rupture of melanomacrophage centers (ANDRÈ *et al.*, 1972). The present study revealed that external and internal signs of tilapia infected with *P. fluorescens* were clearly different from above diseased fishes. The principal lesions observed in diseased tilapia were focal necrosis, abscesses and granulomas. Granulomas usually enclosed neutrophils and affected tissue. Therefore, abscesses and focal necrosis were considered to be initially produced with bacterial invasions and they were subsequently replaced by granulomas. These histopathological aspects were not observed in other fishes infected with *P. fluorescens*.

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**Explanation of Figures**

**Fig. 3.** Exophthalmic eye showing suppurative inflammation. The inflammation extends in the orbital adipose tissue involving the optic nerve and the oculo-motor musculature. H-E, ×10

**Fig. 4.** High power view of the above eye. Bacteria multiply in amorphous matter in the suppurative lesion. Giemsa, ×600

**Fig. 5.** Exophthalmic eye showing granulomatous inflammation. Granuloma encloses infected and inflammed tissue in the orbital adipose tissue. H-E, ×10

**Fig. 6.** Spleen showing abscesses. H-E, ×50

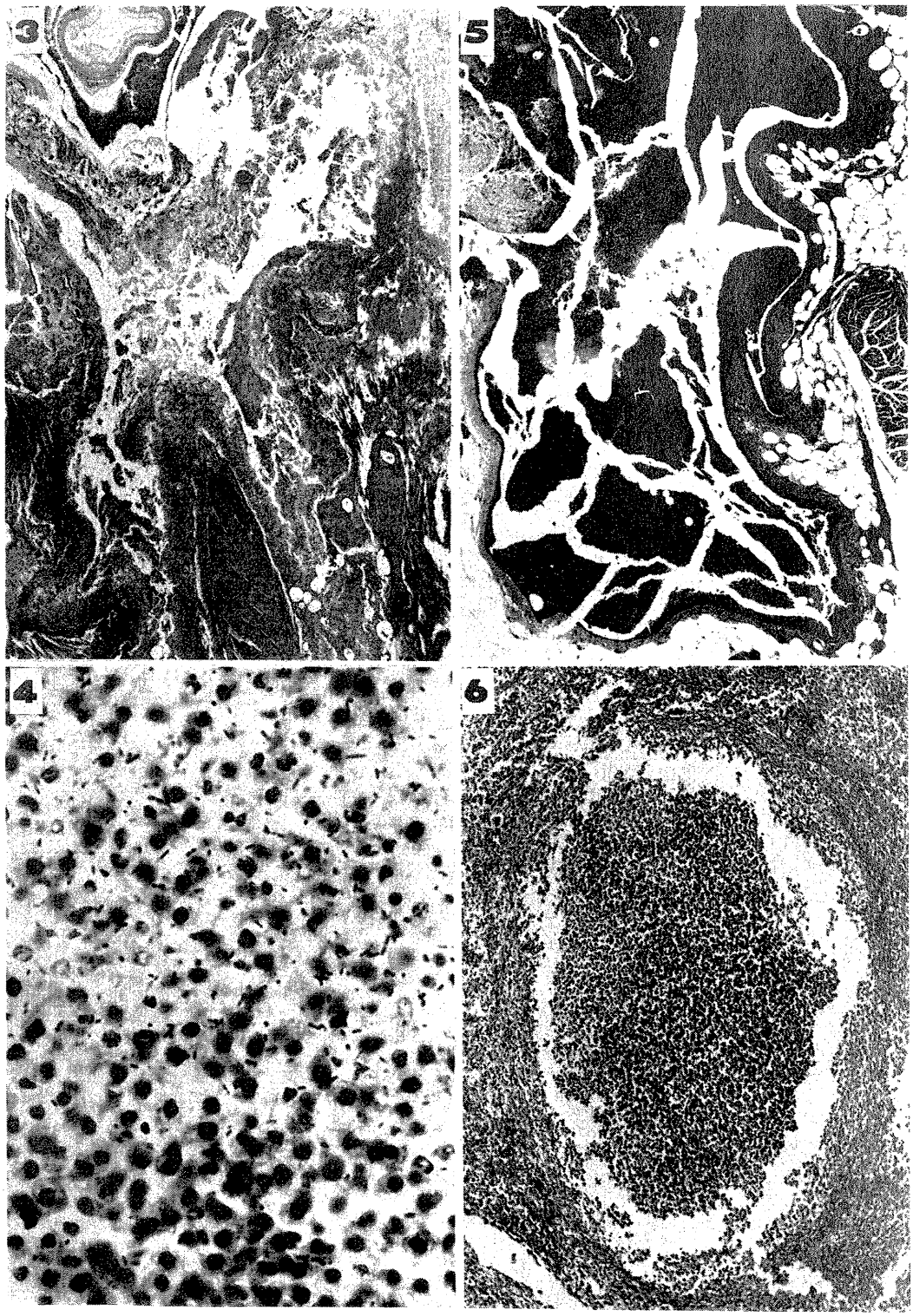
**Fig. 7.** Spleen showing granulomas. H-E, ×160

**Fig. 8.** Liver showing focal necrosis with fibrin precipitation. H-E, ×160

**Fig. 9.** Kidney showing focal necrosis with fibrin precipitation in the hemopoietic tissue. H-E, ×160

**Fig. 10.** Gill filament showing necrosis of the connective tissue with fibrine precipitation and epithelial hyperplasia. H-E, ×100.

**Fig. 11.** Swim-bladder showing granuloma formation. H-E, ×50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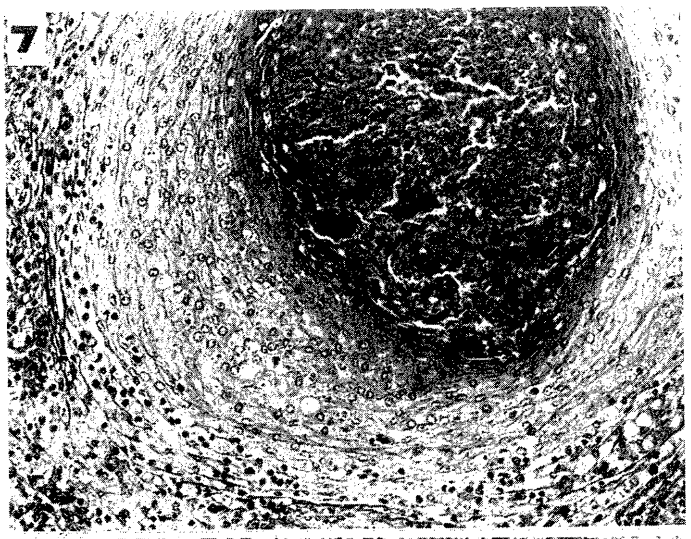
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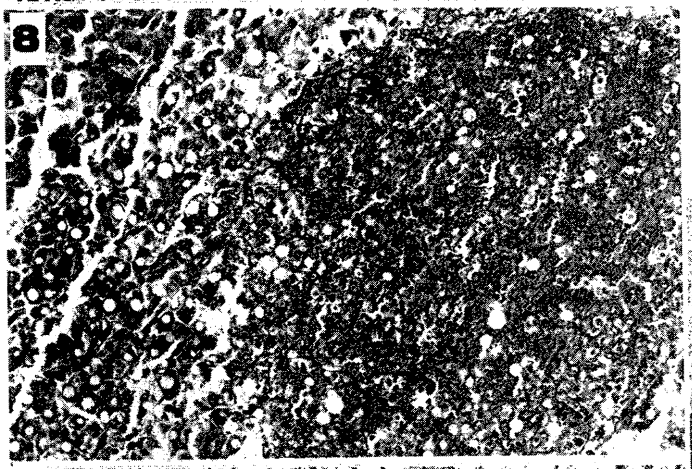
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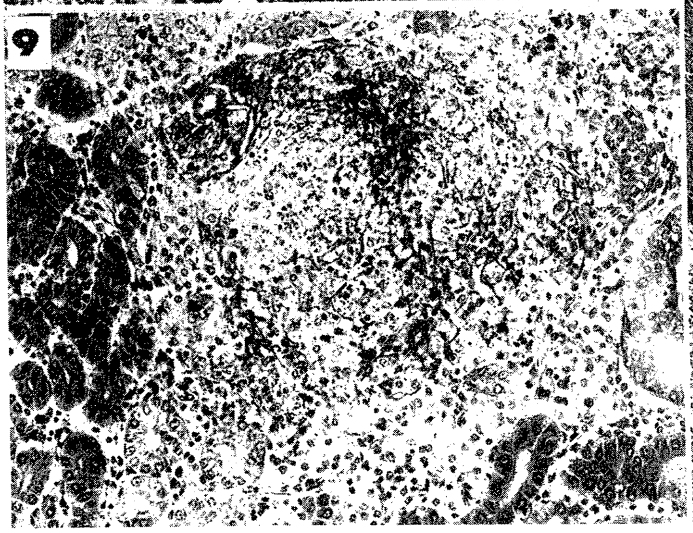
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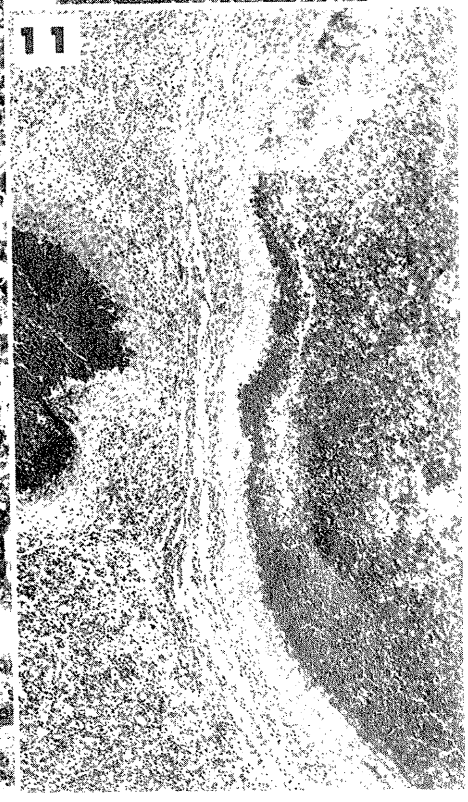
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