Pathological Study on the Scuticociliatosis Affecting Farmed Japanese Flounder (*Paralichthys olivaceus*) in Japan

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ABSTRACT. Pathological findings associated with scuticociliatosis in farmed Japanese flounder in Japan are described. Ten moribund fishes, farmed in Tottori Prefectural Fisheries Experimental Station, showed cutaneous ulcers, darkened skin, fin and tail rot, exophthalmia and alterations in swimming behaviour. Histopathologically, severe epidermal degeneration and necrosis, hyperplasia of branchial epithelium, myositis, myelitis, encephalitis associated with heavy accumulation of scuticociliates in the periorbital cavity and optic nerve fiber were observed. Moreover, masses of ciliates were found to feed on the host tissues such as skeletal muscles, gills and brain, causing severe degenerative changes associated with abundant neutrophilic and lymphocytic infiltration. These findings suggest that the present scuticociliate, *Miamiensis avidus*, is a highly invasive and destructive pathogen infecting Japanese flounder and capable of developing systemic fatal infection.

KEY WORDS: histopathology, *Miamiensis avidus*, *Paralichthys olivaceus*, scuticociliatosis.

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Scuticociliates are free-living in sea-water, and typically scavengers that feed on suspended particulate matters but, under certain circumstances, these ciliates may behave as opportunistic histophagous parasites and actively feed on cells of certain mollusks, crustaceans and fishes, and continue to live and reproduce within the host tissues [1]. Infections by histophagous scuticociliates (scuticociliatosis) have become one of the most important worldwide parasitological diseases in the intensive marine culture of flatfish species including Japanese flounder (*Paralichthys olivaceus*) [6].

A variety of scuticociliates have been isolated and/or detected in diseased fish. For example, *Philasterides dicentrarchi* causes scuticociliatosis in turbot (*Scophthalmus maximus*) [5]. Other pathogenic scuticociliates infecting farmed aquatic animals are *Uronema nigricans* in southern bluefin tuna (*Thunnus maccoyii*) [11]. These ciliates infect the gills, skin, muscles and visceral organs including the intestine, heart and brain. The disease is frequently observed in Japanese flounder, one of the major farmed fish species in Asian countries including Japan, Korea and China. Etiologic agents of the disease include an unidentified scuticociliate [17], *Uronema marinum* [6], *P. dicentrarchi* [10] and *Miamiensis avidus* [8].

In Japan and Korea, outbreaks of scuticociliatosis have frequently occurred in Japanese flounder [15]. Otodate and Matsusato [13] recorded scuticociliatosis in farmed flounders for the first time in Japan. Morphological observations

and small subunit rRNA gene sequences have shown that the causative agent of scuticociliatosis in the flounder is *M. avidus* [8, 15]. However, detailed pathology on scuticociliatosis occurring in Japan has not yet been reported. In addition, very little is known about the route through which the ciliate invade the host tissue and the evasive mechanism of the scuticociliate against the host cellular defense response.

The aim of the present study is to describe the pathological changes associated with scuticociliatosis caused by *M. avidus* in farmed Japanese flounder in Japan.

Ten moribund farmed flounders (total body length ranged from 12 to 17 cm) were obtained from Tottori Prefectural Fisheries Experimental Station in Japan (Table 1). These fishes were collected during an outbreak of scuticociliatosis in July 2005; the mortality of fishes reached 70–80%. Six thousands fishes reared in one indoor reinforced fiberglass tank supplied with 16.5°C underground water were moved to 75 ton round concrete tank supplied with 22°C filtered seawater. Then, antibacterial drug was added to the tank as a prophylaxis against *Flexibacter maritimus*. Three days later, the clinical signs of scuticociliatosis began to appear in all ages of the fishes in the tank. The affected fishes showed alteration in swimming behaviour, darkened skin, a dark patch at the base of the operculum and areas of depigmentation and exophthalmia (Fig. 1), as well as fin and tail rot and skin ulcers surrounded by a whitish halo especially on the abdomen. The entire body of the obtained fishes were preserved in 10% formalin and then transported to Veterinary Pathology Laboratory at Tottori University.

After the gross examination, the skin, gills, eyes, brain, anterior spinal cord, muscles and internal organs were collected and fixed in 10% neutral buffered formalin, pro-

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Table 1. Summarized pathological findings in the fishes affected with scuticociliatosis

Case number	Body length (cm)	Macroscopic findings	Histological findings									
				Skin	Gills	Eye	Muscle	Gastro-intestinal tract	Liver	Kidney	Brain	Spinal cord
1	13	Skin ulcers	Lesions	+	+	–	+	+	–	–	+	+
		Exophthalmia	Parasite	+	+	–	+	+	–	–	+	+
		Dark skin										
2	12	Fin rot	Lesions	–	+	+	+	+	–	+	+	–
		Dark skin	Parasite	–	–	+	+	+	–	+	+	–
		Exophthalmia										
3	13.8	Dark skin	Lesions	–	+	–	+	–	–	+	–	–
		Tail rot	Parasite	–	–	–	+	–	–	+	–	–
		Depigmentation areas										
4	12.6	Dark patch at the operculum base	Lesions	+	+	+	+	+	–	–	–	+
		Depigmentation areas	Parasite	+	–	+	+	+	–	–	–	+
		Exophthalmia										
5	14	No	Lesions	–	–	–	+	–	–	+	+	–
			Parasite	–	–	–	+	–	–	+	+	–
6	16	Dark skin	Lesions	–	–	–	+	+	–	–	+	–
		Exophthalmia	Parasite	–	–	–	+	+	–	–	+	–
7	14.6	No	Lesions	–	+	+	+	+	–	–	–	–
			Parasite	–	+	+	+	–	–	–	–	–
8	15.3	No	Lesions	–	+	+	+	+	–	–	+	+
			Parasite	–	+	+	+	–	–	–	+	+
9	17	Fin rot	Lesions	+	–	–	+	–	–	+	–	–
			Parasite	+	–	–	+	–	–	–	–	–
10	14.5	No	Lesions	–	+	–	+	–	–	–	–	–
			Parasite	–	–	–	+	–	–	–	–	–

cessed, embedded in paraffin wax by a routine method, and sliced into 2–5 μm sections. Sections were then stained with hematoxylin and eosin (H E) and examined microscopically.

Histologically, masses of ciliates were found feeding the host tissues, such as muscles, gills and brain, causing focal degeneration and necrosis. In the ulcerated skin, losses of epidermal epithelial cells associated with inflammation of the underlying hypodermis and musculature were observed (Fig. 2). Degeneration and necrosis of the gill was observed. Scuticociliates along with mononuclear inflammatory cells infiltration were observed in and between the gill filaments and in the gill lamellae; these lesions were associated with hyperplasia of the branchial epithelium. The extensive proliferation of the gill epithelial cells in fusion with the gill filaments was observed with clubbing appearance (Fig. 3). Skeletal muscles showed severe degenerative and necrotic changes together with presence of the scuticociliate in and between the muscle bundles with heavy lymphocytic infiltration (Fig. 4). Ciliates containing numerous erythrocytes in the cytoplasm were observed in the hemorrhagic lesions in the skeletal muscle. Scuticociliates along with mononuclear cells infiltration was also observed in the lamina propria of the gastrointestinal tract. No

inflammatory changes were observed in the liver and spleen. Heavy accumulation of the scuticociliate was observed in the periorbital cavity, optic nerve fiber (Fig. 5), meninges, brain and spinal cord (Fig. 6). Scuticociliates were occasionally observed inside and around the blood vessels in the brain parenchyma.

Scuticociliates, previously regarded as exclusively free-living ciliates, have been recognized as a serious pathogen [3]. Several species of scuticociliates including *M. avidus* are known to be histophagous parasites causing severe infection in Japanese flounder [8, 9] and can even exist in an aquarium rearing no fishes. Pathology of fishes affected with *Uronema* and *Miamiensis* has been reported to be similar to the present cases [4, 12]. Moreover, it was reported that *P. dicentrarchi* and *M. avidus* are conspecific based on the careful morphological description and a wide range of reference comparisons [9]. Although, a detailed morphological study of the present ciliate is beyond the scope of this paper, some interesting findings were observed. The observed scuticociliate was generally elongated with a rounded posterior and bluntly pointed anterior characterized by the shape of a tear-drop; the findings strongly resemble those of the *Uronematid* ciliates, *U. marinum* and *M. avidus*

Fig. 1. Skin shows a dark patch at the base of the operculum (a consolidated arrow), areas of depigmentation (an unconsolidated arrow) and exophthalmia (an arrow head). Skin, Fish No. 4.

Fig. 2. Loss of epidermal epithelial cells (an arrow) with inflammation of the underlying hypodermis and musculature. Ulcerated skin, Fish No. 1, Bar=60 μ m.

Fig. 3. Degeneration and hyperplastic changes with clubbing appearance (an arrow) of the branchial epithelium. Gill, Fish No. 1, Bar=30 μ m.

Fig. 4. Severe degeneration and necrosis of the striated muscle and presence of scuticociliates in and between the muscle bundles. Muscles, Fish No. 7, Bar=30 μ m.

Fig. 5. Scuticociliates (arrow heads) associated with inflammatory cell infiltration are observed inside the optic nerve fiber. Optic nerve, Fish No. 4, Bar=30 μ m.

Fig. 6. Scuticociliates (arrow heads) are observed in the spinal cord. Anterior spinal cord, Fish No. 4, Bar=30 μ m.

[4, 12]. PCR analysis of the ciliate obtained from the fishes suggests that the present scuticociliate is *M. avidus* (data prepared for submission).

In the present fishes, macroscopic skin lesions including cutaneous ulcers and dark patches were observed; these findings are similar to those reported in turbot [5], Japanese flounder [7, 9] and in silver pomfret in Kuwait [2]. Exophthalmia has not been reported in Japanese flounder.

In the present study, histological analysis of the affected fishes showed masses of ciliates feeding on the host tissues such as muscles, gills and brain, causing degenerative and necrotic changes. Severe epidermal degeneration and necrosis, myositis, encephalitis and myelitis were the most frequent changes in the present study. These histological findings are in accordance with those reported in marine fishes [3], turbot [4], Japanese flounder [6, 7, 9] and in silver pomfret [2]; skeletal muscle was the most severely damaged among the tissues affected; suggesting that the muscle is the most predisposed to the infection [3]. Ciliates containing numerous erythrocytes in the cytoplasm were observed in the hemorrhagic lesions in the skeletal muscle [2, 6]. Heavy accumulation of scuticociliates in the periorbital cavity and optic nerve fiber was also observed in this study.

Environmental stress is believed to play an essential role for the occurrence of scuticociliatosis in a variety of marine water fishes held in aquarium [3]. Stress factors including sudden increase in the water temperature and/or antibiotic exposure may be considered as triggers for the present outbreak of the scuticociliatosis [3, 11].

The presence of ciliates in the gill, skin, skeletal muscle and blood vessel suggests that the initial route of entry of the parasite could be the skin and/or gill lesions and its subsequent spread into the body was perhaps *via* blood vessels [5, 7, 14, 16]. Scuticociliate may also enter through the periorbital route and migrate through the optic nerve to the brain [14]; this would explain the occurrence of the severe brain lesions in association with exophthalmia with abundant ciliates in the periorbital cavity in this study.

Based on these pathological findings, it is elucidated that the present scuticociliate is a highly invasive and destructive pathogen to the host tissue and the scuticociliate could achieve rapid expanse inside the host organs and tissues.

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