Jpn. J. Vet. Res., **35**, 227–234 (1987)

BACTERIAL INFECTION WHICH PRODUCES HIGH MORTALITY IN CULTURED JAPANESE FLOUNDER (*PARALICHTHYS OLIVACEUS*) IN HOKKAIDO

Hiroshi KODAMA, Takashi MURAI, Yuki, NAKANISHI, Fukie YAMAMOTO, Takeshi MIKAMI and Hisao IZAWA

(Accepted for publication August 6, 1987)

An infectious disease occurred in two groups of young Japanese flounder (*Paralichthys olivaceus*) reared in a commercial fish farm in Hokkaido in August 1985. These fish had been introduced in January 1985 from individual fish farms located in the southwestern part of Japan. More than 80% of the fish died within two weeks. Ulcerative lesions and loss of the skin were prominently observed, and the muscle was exposed. Hemorrhage and loss of fins, protrusion of the rectum, and swelling of the spleen were also observed. *Edwardsiella tarda* was isolated in pure culture from various tissues and organs. Japanese flounder inoculated with the isolate experimentally by infiltration or by an intraperitoneal route died one week after the inoculation. Swelling of abdomen and accumulation of ascitic fluid containing blood were significant.

Key words: *Edwardsiella tarda*, edwardsiellosis, Japanese flounder

INTRODUCTION

In the past several years, cultivation of the Japanese flounder (*Paralichthys olivaceus*) has developed, especially in the southwestern part of Japan. Since attempts to grow Japanese flounder in cold water areas such as Hokkaido have also been made, it is concerned about the occurrence of infectious diseases among these fish populations. We encountered an outbreak of an infectious disease that produced high mortality among populations of young Japanese flounder in a commercial fish farm in Hokkaido. *Edwardsiella tarda* was isolated from various tissues and organs of the dead fish. Gross lesions observed in fish, however, indicated that the fish were dually infected with *E. tarda* and other species of bacteria such as gliding bacteria. The significance of the bacterial infection in the cultured Japanese flounder populations in cold water areas is discussed.

* Department of Epizootiology, Faculty of Veterinary Medicine, Hokkaido University, Sapporo 060, Japan

MATERIALS AND METHODS

Fish: Japanese flounder which died during the outbreak of the disease (ten months old, weighing 40±10g, total length 16±1cm) were used for the isolation of bacteria.

Japanese flounder (weighing 160±90g) were also supplied through the courtesy of Sun-Piazza Aquarium, Sapporo. These fish were used for experimental inoculation of the bacteria isolated in the present outbreak. The fish were raised in our laboratory in a glass sea-water aquarium (160 liters) equipped with thermo-regulating and filtration systems (water temperature 20°C). They were fed daily with minced frozen sand lance (*Ammodytes personatus*).

Isolation and characterization of bacteria: Commonly used biochemical tests were employed for the identification of bacteria. The characteristics of the bacteria isolated from dead and moribund fish were compared with those of *E. tarda, Edwardsiella hoshinae* and *Edwardsiella ictaluri* listed in BERGEY'S Manual of Systematic Bacteriology.[2)]

A drug sensitivity test was done by the disk method (Showa Disk, Showa Yakuhin, Tokyo, Japan).

Experimental inoculation: To observe the pathogenicity of the isolate to the Japanese flounder, experimental inoculation was performed. No becterium was isolated from visceral organs of normal fish when cultivated on brain heart infusion agar. Three fish were immersed for 30 minutes in water containing 4.2×10^8 cells/ml of *E. tarda*. Another two fish were inoculated intraperitoneally with 1.7×10^6 bacteria. The fish were observed for two weeks for symptoms of the disease and for mortality.

RESULTS

Case history: Fry of Japanese flounder were introduced to the present fish farm, which faces the Sea of Japan, in January 1985. The fry were from two different districts in Honshu (1,000 from a fish farm in Kagawa prefecture; group I, and 1,000 from a fish farm in Wakayama prefecture; group II). They were separately reared in five round plastic aquaria (diameter 3 m, depth 1m; 300 to 500 fish in each aquarium) placed on the ground. Aquaria were supplied with sea water with aeration. Fish were fed with minced sand lance.

In August, a sudden increase in the deaths of fish was noticed among group I. Water temperature was 18°C when the outbreak occurred. About 800 fish died within two weeks of the outbreak. The disease spread to another group of fish two weeks later, and the mortality reached approximately 90%.

The most remarkable gross lesions were ulcerative changes of the skin. In the heavily affected fish, there was extensive loss of epidermis and the muscle was exposed (Fig. la). The ulcerative lesions were observed only in the superior part of the body but not in the inferior part. Most of the affected fish also showed

hemorrhage in the fins, which were partially missing (Fig. 1b). Hemorrhage was also observed in the eyes and around the oral cavity. Protrusion of the rectum was noticed in some of noticed in some of the dead fish. There was no marked swelling of the abdomen or accumulation of ascitic fluid. At necropsy, swelling of the spleen was noticed.

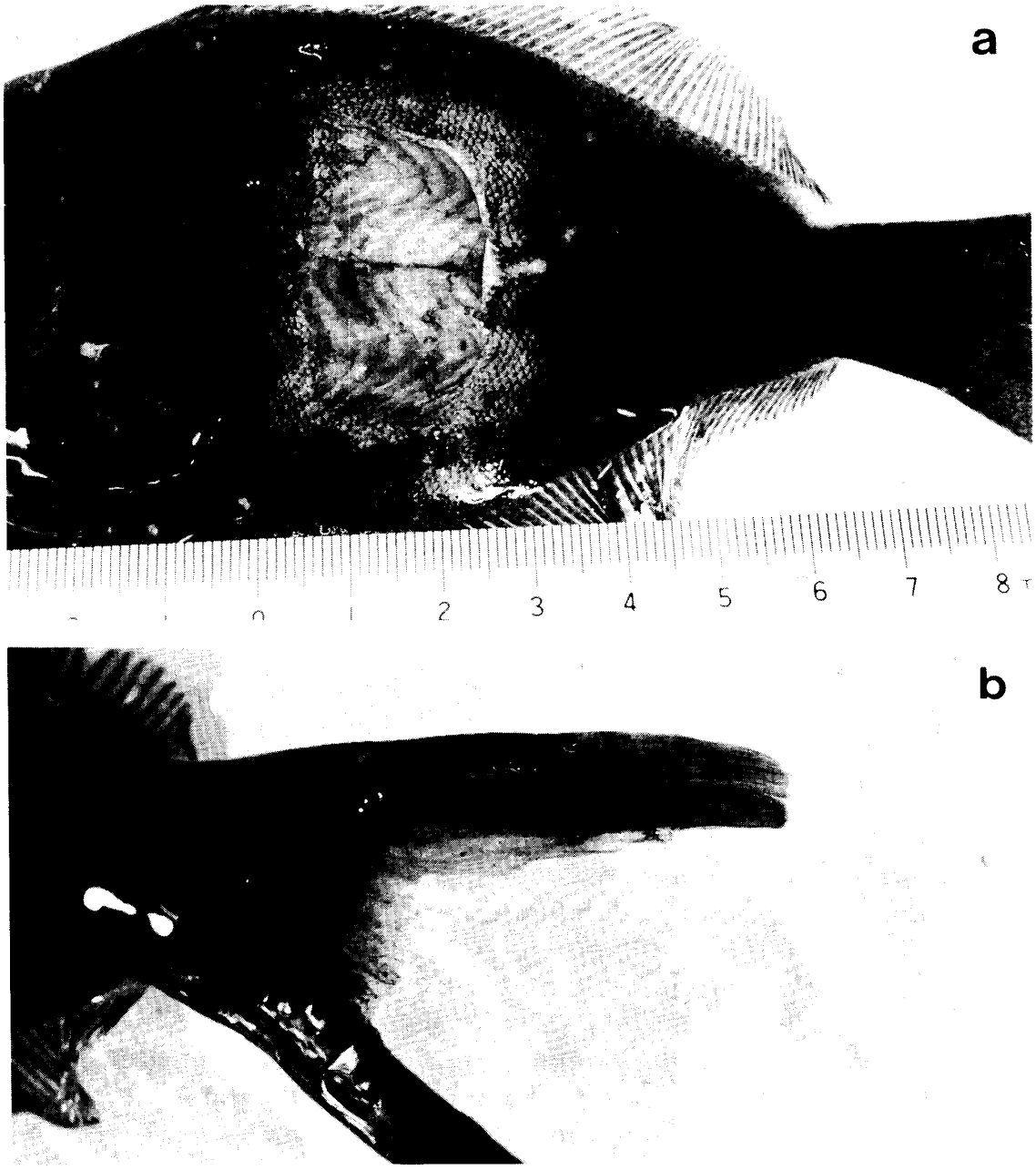

FIGURE 1 Gross lesions observed in Japanese flounder. (a) Extensive ulcerative lesion on the skin. (b) Partial disappearance of caudal fin and loss of caudal softrays.

Isolation and characterization of bacteria: Mucus from the skin lesions and gills was taken, streaked onto glass slides, and observed under microscopy. No filamentous bacteria showing active motility were observed. In the present experiment, we did not perform virological examinations since we did not maintain line cells derived from salt water fish.

Internal organs of the dead fish were taken aseptically and were streaked onto heart infusion agar containing 1.5% NaCl. Specimens of skin lesions were also cultivated at 23°C for two days. One bacterium was isolated in pure culture from all of the samples cultivated. The isolate was identified as *E. tarda* (Table 1). The bacteria grew between the temperatures of 20 and 37°C in peptone water containing 1.5% NaCl, but did not grow at 10 or 41°C (Fig. 2a). The optimum temperature for the growth of the bacteria was 25°C. The bacteria grew in medium containing NaCl at the concentration of 0.5 to 6%, but did not grow in medium containing 0 or 10% NaCl (Fig. 2b).

The isolate was sensitive to penicillin, oxytetracycline, tetracycline, streptomycin, chloramphenicol and furazolidone, but was resistant to ampicillin, colistin and sulfadimethoxine.

Experimental inoculation: Two out of three Japanese flounder inoculated by infiltration and both of those inoculated by an intraperitoneal route died within seven to eight days after the inoculation with the present isolate of *E. tarda*. The fish showed sluggishness and anorexia. They exhibited signs of anguish such as increased respiration rates and bending the body backwards. Hemorrhage occurred around the oral cavity and on the fins, but ulcerative lesions of the skin were not observed. Swelling of the abdomen was noticed and ascitic fluid containing blood accumulated in the body cavity. Swelling of the spleen and the liver, hemorrhagic enteritis and peritonitis were also significant. The *E. tarda* was recovered in pure culture from various visceral organs and tissues of the dead fish.

DISCUSSION

Since HOSHINA[4] first reported edwardsiellosis in Japanese eel (*Anguilla japonica*), *E. tarda* infections have been found among cultured red sea bream (*Pagrus major*; referred to by MIYAZAKI & KAIGE[10]), Japanese flounder, [11,14] tilapia (*Tilapia nilotica*),[8] goldfish (*Carassius auratus*)[7] and carp (*Cyprinus carpio*)[12] in Japan. In the United States, edwardsiellosis in channel catfish (*Ictalurus punctatus*) is caused by *E. ictaluri*[3] as well as *E. tarda*.[9] The cases of edwardsiellosis reported in Japan all occurred in the summer in the southwest part of Japan. It is generally thought that the occurrence of edwardsiellosis is influenced by water temperature. Previous reports indicated that the outbreaks occurred at water temperatures of 20 to 30°C in eel,[13] 22 to 24°C in Japanese flounder[11] and 24 to 29°C in channel catfish.[5] The water temperature of the Sea of Japan is usually below 20°C even in midsummer. In

TABLE 1 Main characteristics of *Edwardsiella tarda* isolated from Japanese flounder

	Present isolate	T1	T2	H	I
Morphology	rod	rod	rod	rod	rod
Gram staining	−	−	−	−	−
Motility	+	+	+	+	−
Growth on MacConkey agar	−				
Hemolysis (sheep red blood cells)	−				
Pigment production	−	−	−	−	−
Luminescence	−				
Sensitivity to O/129	−				
Catalase	−				
Oxidase	−	−	−	−	−
OF test	fermentative				
Hydrogen sulfide	+	+	−	−	−
Indole production	+	+	+	−	−
MR test	+	+	+	+	−
VP test	−	−	−	−	−
Nitrate reduction	+	+	+	+	+
Urease	−	−	−	−	−
Casein hydrolysis	+				
Gelatin hydrolysis	−	−	−	−	−
Lysine decarboxilase	+	+	+	+	+
Citrate utilization (Simmons')	−	−	−	−	−
Gas from glucose	+	+	−	−	d
ONPG	−	−	−	−	−
Malonate utilization	−	−	−	+	−
Esculin hydrolysis	+	−	−	−	−
Starch hydrolysis	+	−	−	−	−
Acid from arabinose	−	−	+	−	−
lactose	−	−	−	−	−
maltose	+				
mannitol	−	−	+	+	−
raffinose	−	−	−	−	−
salicin	+	−	−	d	−
sorbitol	−				
sucrose	+	−	+	+	−
trehalose	+	−	−	+	−
xylose	−	−	−	−	−

T1 ; *Edwardsiella tarda* wild-type, T2 ; *E. tarda* biogroup 1, H ; *Edwardsiella hoshinae*, and I ; *Edwardsiella ictaluri*. d unfixed.

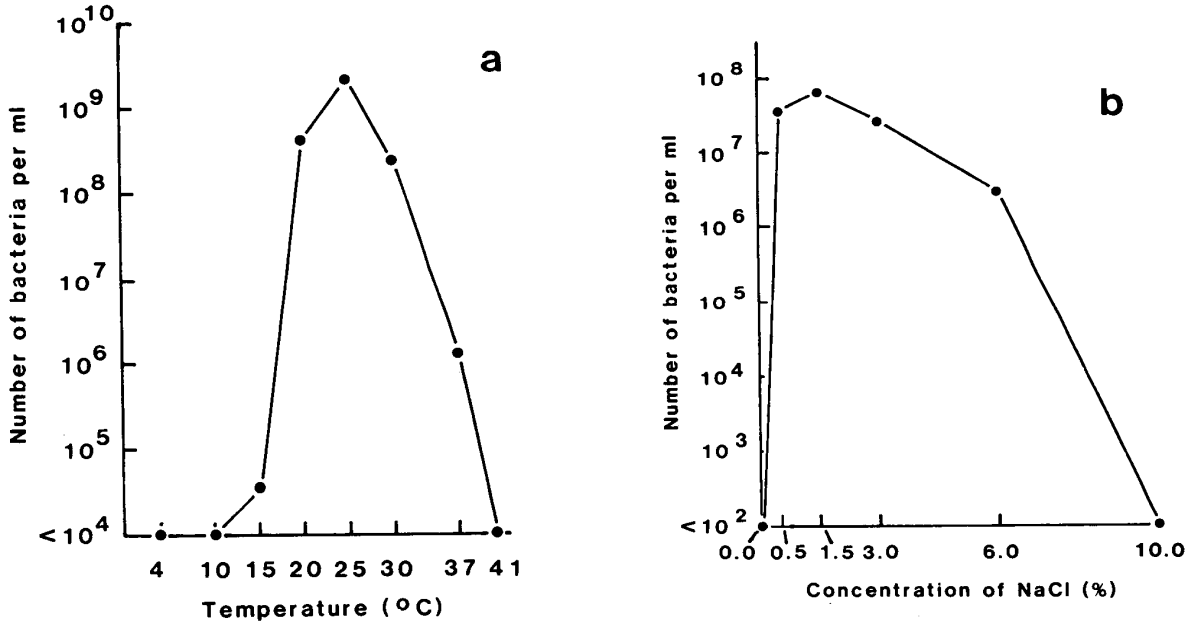

FIGURE 2 Growth of *Edwardsiella tarda* isolated from Japanese flounder at various temperatures and in media containing various concentrations of NaCl. (a) The bacteria were cultivated at 4 to 41°C in peptone water containing 1.5% NaCl for 48 hours. (b) The bacteria were cultivated in peptone water containing 0 to 10% NaCl at 25°C for 48 hours.

the present outbreak, Japanese flounder were reared at the water temperature of 18°C when the disease occurred and, therefore, it appears to be a rather unusual case of edwardsiellosis.

In the present outbreak, the course of the disease was acute and more than 80% of the fish died within two weeks. The major gross lesions observed in fish were the ulceration and loss of skin, hemorrhage, and the disappearance of fins. Swelling of the abdomen and accumulation of ascitic fluid reported previously[10,11] were not prominently observed in the present case. When Japanese flounder were infected experimentally with *E. tarda* by intraperitoneal inoculation or by immersion, they died seven to eight days after the inoculation. In this case, lesions in the skin and fins were not prominent, but swelling of the abdomen and the accumulation of ascitic fluid were observed in fish infected by both of the inoculation routes. BAXA et al.[1] recently reported the pathogenesis of gliding bacteria isolated from diseased cultured flounder. Gross lesions in fish reported by them appeared to be similar to that observed in the present outbreak. Though filamentous bacteria showing gliding motility were not observed in skin lesions under microscopy, and the attempt to isolate the gliding bacteria was not done in the present study, it is possible that the fish had been also infected with gliding bacteria.

Since we did not perform virological examinations in the present study, the participation of viral infection as a cause of the present outbreak can not be completely ruled out. Rhabdovirus infection in Japanese flounder was reported recently by KIMURA et al.[6] They wrote that the disease occurred in the winter when the water temperature was 2 to 5°C. The common gross lesions were congestion of the gonads and hemorrhage in the skeletal muscle and fins. Therefore, the present outbreak seemed to differ from the rhabdovirus infection.

Since the culture of Japanese flounder and warm water fishes such as tilapia and eel have been introduced to Hokkaido, there is concern about the outbreak of new infectious diseases which have not existed in cold water areas. Such diseases may be introduced by fishes which are infected with pathogenic organisms. They spread among fish populations via these outbreaks and contaminate the environmental water and, therefore, it is important to avoid the introduction of carriers from polluted areas, to treat of fish with antibiotics before transportation, and to prevent the spread of pathogens to the environment by means of such methods as sterilization or purification of sewage.

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