

情 報

A Case of *Edwardsiella tarda* Infection in Cultured Colored Carp *Cyprinus carpio*

Darunee SAE-OUI, Kiyokuni MUROGA and Toshihiro NAKAI

Faculty of Applied Biological Science, Hiroshima University, Fukuyama 720, Japan

(Received June 19, 1984)

From the end of August to late in October 1983, an epizootic occurred among 60 colored carp *Cyprinus carpio* in a garden pond of a businessman in Mihara city, Hiroshima Prefecture. Affected fish exhibited hemorrhage and erythema on the body surface and fins. From the kidney, spleen and liver of moribund fish, *Edwardsiella tarda* was purely isolated, and the isolates were confirmed to cause mortality not only in carp but also in Japanese eel *Anguilla japonica*. This is the first recorded case of edwardsiellosis in carp.

Edwardsiella tarda has been isolated from wide variety of animals and implicated in disease in animals including human. It has also been reported as the disease agent from various fishes, not only freshwater fishes but also marine fishes. In the United States, it is known as a causative agent of emphysematous putrefactive disease in channel catfish *Ictalurus punctatus* (MEYER and BULLOCK, 1973). It was also isolated from a diseased wild large-mouth bass *Micropterus salmoides* (WHITE *et al.*, 1973) and chinook salmon *Oncorhynchus tshawytscha* (AMANDI *et al.*, 1982). In Japan, it has been reported to cause epizootics in cultured eels *Anguilla japonica* (HOSHINA, 1962; WAKABAYASHI and EGUSA, 1973), goldfish *Carassius auratus* (KOU and EGUSA, 1968), tilapia *Sarotherodon niloticus* (KITAO *et al.*, 1980*) and flounder *Paralichthys olivaceus* (NAKATSUGAWA, 1983), and wild mullet *Mugil cephalus* (KUSUDA *et al.*, 1976) and akame *Liza akame* (MUROGA, 1979). Non-motile strains of *E. tarda* were isolated from crimson seabream *Eynniss japonicus* (KUSUDA *et al.*, 1977), red seabream *Chrysophrys major* and yellowtail *Seriola quinqueradiata* (YASUNAGA *et al.*, 1982). In this report, a case of edwardsiellosis in

colored carp *Cyprinus carpio* is presented because the infection has not been reported yet in this fish species.

Materials and Methods

From the end of August to late in October 1983, an epizootic occurred among 60 colored carp in a garden pond of a businessman in Mihara city, Hiroshima Prefecture. During the two months, fish died by twos and threes and finally became extinct. Diseased fish exhibited sparse hemorrhage throughout the body surface. Some showed severe hemorrhage at the lower jaw and a small ulcerative lesion at the dorsal body surface. Erythema was also noticed at pectoral, anal, and caudal fins.

In the middle of October, when water temperature of the pond was 18°C, the kidney, spleen and liver tissues of three moribund fish were streaked on nutrient agar, incubated at 25°C for 24 h, and a bacterial culture was purely obtained. Three isolates were submitted to morphological and biochemical characterization tests.

Pathogenicity of the three isolates to colored carp (10-96 g) and Japanese eel (60-136 g) was tested at 25°C. Doses of 10⁶, 10⁷, 10⁸ CFU/100 g fish and 10⁸-10⁹ CFU/100 g fish of each isolate were intramuscularly injected into each of five carp and each of two eels, respectively.

* KITAO, T., T. AOKI, K. TAWARA, K. KUMADA, K. SHIOMITSU and M. FUKUDOME (1980): On an edwardsiellosis in tilapia. Abstract, Ann. Meet. Japan. Soc. Sci. Fish., (April), p. 82.

Table 1. Morphological and biochemical characteristics of the isolates from carp.

Test	Result
Gram stain	—
Motility	+
Flagella	Peritri.
Catalase	+
Cytochrome oxidase	—
Hydrogen sulfide	+
Urease	—
Indole production	+
Methyl red	+
Voges-Proskauer	—
β -Galactosidase	—
KCN	—
Gelatin	—
Lysine decarboxylase	+
Ornithine	+
Arginine	—
Phenylalanine	—
Citrate (Simmons)	—
Citrate (Christensen)	+
Citrate (KP)	+
d-Tartrate	—
Malonate	—
Gas from glucose	+
Acid from	
Glucose	+
Maltose	+
Lactose	—
Sucrose	—
Mannitol	—
Dulcitol	—
Salicin	—
Adonitol	—
Inositol	—
Sorbitol	—
Arabinose	—
Raffinose	—
Rhamnose	—
Xylose	—
Trehalose	—
Cellobiose	—
Erythritol	—
Esculin	—

Results and Discussion

The isolates grew on Salmonella-Shigella (SS) agar, forming a small transparent colony with black center, and were gram negative, rod shaped, and motile with peritrichous flagella. It was found by characterization tests that the three isolates

Table 2. Pathogenicity for carp and Japanese eel of 3 strains of *E. tarda* isolated from diseased carp. (Intramuscular injection)

Strain	Exp. fish and dose*			
	Colored carp			Japanese eel
	10 ⁸	10 ⁷	10 ⁶	10 ⁵ -10 ⁹
HC-1	5/5**	5/5	2/5	2/2
HC-2	5/5	4/5	1/5	2/2
HC-3	5/5	1/5	5/5	2/2

* Injection dose: cells/100 g body weight.

** Number of fish died/tested.

were identical to each other. According to the morphological and biochemical characteristics shown in Table 1, the organism was identified as *Edwardsiella tarda* (EWING *et al.* 1965). The isolates showed positive agglutination with an antiserum prepared with a strain of *E. tarda* isolated from diseased eels.

Pathogenicity of the isolates is shown in Table 2. The isolates were able to cause mortality not only in colored carp but also in eels. From the results shown above, the disease case occurred in the carp pond was attributed to edwardsiellosis.

Edwardsiellosis is one of the most common and widely occurring diseases in eel farms in Japan. Although it was demonstrated by HOSHINA (1962) that carp was susceptible to *E. tarda* which was isolated from diseased eels, the infection has never been reported from carp before. We could not find any relation between the present case and eel edwardsiellosis because there was no eel farm around the garden pond or the carp hatchery from which the carp were introduced.

Acknowledgements

The authors would like to acknowledge Mr. I. NAKOHGE, the owner of the carp, and Mr. S. KAWAMOTO for their help in sample collection.

References

- AMANDI, A., S. F. HRU, J. S. ROHOVEC and J. L. FRYER (1982): Isolation and characterization of *Edwardsiella tarda* from fall chinook salmon (*Oncorhynchus tshawytscha*). *Appl. Environ. Microbiol.*, **43**, 1380-1384.
- EWING, W. H., A. C. MCWHORTER, M. R. ESCOBAR and A. H. LUBIN (1965): *Edwardsiella*, a new

- genus of enterobacteriaceae based on a new species, *E. tarda*. *Int. Bull. Bact. Nomen. Taxon.*, **15**, 33-38.
- HOSHINA, T. (1962): On a new bacterium, *Paracolobactrum anguillimortiferum* n. sp. *Bull. Japan. Soc. Sci. Fish.*, **28**, 162-164.
- KOU, G. H. and S. EGUSA (1968): A paracolon isolated from eels and goldfish. *Fish Pathol.*, **3**(1), 58-61.
- KUSUDA, R., T. TOYOSHIMA, Y. IWAMURA and H. SAKO (1976): *Edwardsiella tarda* from an epizootic of mullets (*Mugil cephalus*) in Okitsu Bay. *Bull. Japan. Soc. Sci. Fish.*, **42**, 271-275.
- KUSUDA, R., T. ITAMI, M. MUNEKIYO and H. NAKAJIMA (1977): Characteristics of a *Edwardsiella* sp. from an epizootic of cultured crimson sea breams. *Bull. Japan. Soc. Sci. Fish.*, **43**, 129-134.
- MEYER, F.P. and G.L. BULLOCK (1973): *Edwardsiella tarda*, a new pathogen of channel catfish (*Ictalurus punctatus*). *Appl. Microbiol.*, **25**, 155-156.
- MUROGA, K. (1979): Ulcer disease of Akame (Mugilidae) in the estuary of the River Ashida. *Fish Pathol.*, **13**, 163-167.
- NAKATSUGAWA, T. (1983): *Edwardsiella tarda* isolated from cultured young flounder. *Fish Pathol.*, **18**, 99-101.
- WAKABAYASHI, H. and S. EGUSA (1973): *Edwardsiella tarda* (*Paracolobactrum anguillimortiferum*) associated with pond-cultured eel disease. *Bull. Japan. Soc. Sci. Fish.*, **39**, 931-936.
- WHITE, F. H., C. F. SIMPSON and L. E. WILLIAMS, Jr. (1973): Isolation of *Edwardsiella tarda* from aquatic animal species and surface waters in Florida. *J. Wildl. Dis.*, **9**, 204-208.
- YASUNAGA, N., S. OGAWA and K. HATAI (1982): Characteristics of the fish pathogen *Edwardsiella* isolated from several species of cultured marine fishes. *Bull. Nagasaki Pref. Inst. Fish.*, No. 8, 57-65.