Susceptibility of Five Salmonid Fishes to *Renibacterium salmoninarum*

Masahiro Sakai, Shizuo Atsuta
and Masanori Kobayashi

School of Fisheries Science, Kitasato University
Sanriku, Iwate 022-01, Japan

Bacterial kidney disease (BKD) is an infection of salmonid fish caused by a gram-positive diplobacillus classified as *Renibacterium salmoninarum*¹⁾. This disease affects only salmonid fish and the infection of non-salmonid fish has not been reported²⁾. In Japan, BKD was first reported from masu salmon, *Oncorhynchus masou*, chinook salmon, *O. tshawytscha*, sockeye salmon, *O. nerka* and pink salmon, *O. gorbuscha*³⁾ and was rapidly spread to many parts of Japan. However, the susceptibility to the disease among salmonid fishes cultured in Japan has not been compared yet. In this study, the susceptibility to *R. salmoninarum* was compared among rainbow trout, *O. mykiss*, chum salmon, *O. keta*, coho salmon, *O. kisutch*, masu salmon and Japanese charr, *Salvelinus pluvius*.

Materials and Methods

In this study, *R. salmoninarum* KU8503 isolated from coho salmon in Iwate Prefecture in 1985 was used. The bacterium was grown on KDM-C⁴⁾ for 14 days at 15°C. Rainbow trout weighing about 20 g were supplied from Iwate Experimental Fisheries Station, and coho salmon (about 6 g), masu salmon (about 4.2 g) and Japanese char (about 15 g) were received from private fish farms. Chum salmon (about 5 g) was supplied from a chum salmon hatchery in Iwate Prefecture. Ten fish from each fish species were confirmed by an indirect fluorescent antibody test (IFAT)⁵⁾ and an indirect dot blot assay (IDBA)⁶⁾ that *R. salmoninarum* antigen was not detected from these fishes.

Challenge was conducted by the method of Sakai *et al.*⁷⁾ Fish were injected intraperitoneally with 10⁸ or 10⁷ bacterial cells suspended in PBS. After injection, groups of 18–60 fish were maintained at 12°C and the mortalities were recorded daily for 50 days. All moribund fish

were necropsied and kidney smears were stained by Gram. For control, each fish species injected with the same volume of physiological saline (PS) were maintained in the same conditions.

Fifty days after infection, the kidney smears from all surviving fish were prepared on non fluorescent slides, and IFAT was used to detect the *R. salmoninarum* antigen. The heat extract of kidney from all surviving fish was also prepared by the method of Kimura and Yoshimizu⁸⁾, and the presence of *R. salmoninarum* antigen was examined by IDBA.

Results and Discussion

Coho salmon inoculated with 2.0×10^8 cells/fish of *R. salmoninarum* began to die 11 days after inoculation with the number of deaths peaking 16–18 days after challenge. Mortality continued until 35 days after challenge and 21 of the 24 fish died (87.5%). In the case of coho salmon inoculated with 2.0×10^7 bacterial cells, the accumulated mortality was 72.0%. Gram-positive *R. salmoninarum* like cells were observed from all dead coho salmon and following 4 salmonids. Chum salmon injected with 5.7×10^8 cells began to die 7 days after infection and all fish died within 18 days after challenge. For fish injected with 5.7×10^7 cells, the accumulated mortality was 98.1% 35 days after challenge. The mortality of masu salmon was 91.0% in the fish group injected with 1.2×10^8 *R. salmoninarum* cells, and 53.0% for 1.2×10^7 cells. In the group of rainbow trout injected with 4.8×10^8 cells, the fish began to die 18 days after challenge with the number of mortalities peaking 27–30 days after challenge. In this group, 94.4% of the fish died. In the group injected with 4.8×10^7 cells, mortality was only 15%. In the case of Japanese char, the mortality was 80.0% in the group injected with 1.2×10^8 cells and 16% in 1.2×10^7 cells.

The IFAT detected *R. salmoninarum* antigen from kidney smears of all surviving fish (Table 1). The same results were indicated by the IDBA.

Sanders *et al.*⁹⁾, who compared the susceptibility of sockeye salmon, coho salmon and steelhead trout to *R. salmoninarum*, concluded

Table 1. Susceptibility of coho salmon, chum salmon, masu salmon, rainbow trout and Japanese char against *Renibacterium salmoninarum*

Fish species	Challenge dose (cells/fish)	Number of fish (N)	Number of dead fish (Mortality %)	Number of surviving fish with <i>R. salmoninarum</i> antigen		
				Gram stain	IFAT ¹⁾	IDBA ²⁾
Coho salmon	2.0×10^8	24	21 (87.5)	0	3	3
	2.0×10^7	25	18 (72.0)	0	7	7
Chum salmon	5.7×10^8	40	40 (100)	0	0	0
	5.7×10^7	40	39 (98.1)	0	1	1
Masu salmon	1.2×10^8	65	59 (91)	0	6	6
	1.2×10^7	60	32 (53)	0	18	18
Rainbow trout	4.8×10^8	18	17 (94.4)	0	1	1
	4.8×10^7	20	3 (15)	0	17	17
Japanese char	1.2×10^8	25	20 (80)	0	5	5
	1.2×10^7	25	4 (16)	0	21	21

IFAT¹⁾: Indirect Fluorescent Antibody Test.IDBA²⁾: Indirect Dot Blot Assay.

that sockeye salmon was the most sensitive to *R. salmoninarum* and steelhead trout was less sensitive. Kawamura *et al.*¹⁰⁾ observed that chum salmon was the most sensitive to this disease in experimental transmission from diseased masu salmon to chinook salmon, masu salmon, chum salmon and steelhead trout. Kaattari *et al.*¹¹⁾ compared the sensitivities of chinook salmon, coho salmon and rainbow trout to *R. salmoninarum* and discussed the relationship between susceptibility and serum antibody titers against *R. salmoninarum*. Rainbow trout immunized with heat-killed and soluble *R. salmoninarum* antigen emulsified in Freund's complete adjuvant produced a much greater antibody response, followed by coho salmon and chinook salmon.

In this study, chum salmon was most susceptible to *R. salmoninarum*, and rainbow trout and Japanese char were less susceptible. However, there remains a possibility that the difference in susceptibility may be caused by the size of the fish, for rainbow trout and Japanese char used in this experiment were larger than the other fishes.

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