

The Causative Agents of Mortalities of Pen-Cultured Coho Salmon, *Oncorhynchus kisutch*, in Japan

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Abstract - The causes of mortalities of pen-cultured coho salmon, *Oncorhynchus kisutch*, were investigated using bacterial and histopathological procedures.

Three main causative agents were identified: BKD, vibriosis and a nutrient-deficiency disease represented by lipid degeneration of the liver. Some 42% of the fish examined were BKD-positive by the dot blot assay; the proportion containing *Vibrio anguillarum* was 12%; 66% had lipid degeneration of liver. Although 12% of the fish did not have any of these three agents, they did show some degeneration of the liver. The results suggested that the primary disease in pen-cultured coho salmon was a nutritional disease caused by insufficient feeding and this stress induced infectious diseases such as BKD or vibriosis.

In Japan, the production of coho salmon, *Oncorhynchus kisutch*, reached 16,000 t in 1989. Eggs of coho salmon are imported from the USA and Canada. After hatching, fry are reared in freshwater for 10 months, before transfer to seawater cages where they grow to about 2 kg before harvesting. The fish are kept in seawater from November to July. In pen-cultured coho salmon, furunculosis (Ezura et al. 1984), vibriosis (Tajima et al. 1981; Kurose et al. 1989) and BKD (Kimura and Yoshimizu 1981; Sakai et al. 1989) have already been reported. Recently, Atsuta et al. (1988) indicated that the liver of many coho salmon suffered from lipid degeneration caused by insufficient food. In this study, the frequency of this nutritional disease and its relation with other diseases were examined.

Diseased or moribund coho salmon kept in seawater were collected from seven fish farms in Iwate Prefecture in April 1988. One hundred and one fish samples were collected with mean body weight 1.2 kg. Kidney samples were inoculated on Trypto-soya Agar (Nissui), 0.5% NaCl was added and they were incubated at 20°C for 2 days.

Morphological and biochemical characteristics of the isolated strains were examined according to the standard methods described in Cowan and Steel's manual (Cowan 1974). In the strains of *Vibrio anguillarum*, the rapid slide agglutination test with A, B, C and D type antisera was used. Kidney samples used for the detection of BKD were fixed in 10% formalin solution and processed for histopathological studies. Paraffin-embedded sections (5-7 μm thick) of the tissues were stained with hematoxylin and eosin. Frozen tissue sections (10 μm thick) of liver were also prepared and stained by Sudan IV, Ziehl-Neelsen's or Nile blue methods.

Bacteria were isolated from 18 samples (18%) from four fish farms. All isolated bacteria were classified as belonging to the genus *Vibrio*. In the rapid slide agglutination test, all bacteria agglutinated with C-type antiserum of *Vibrio anguillarum*. Based on these characteristics, all isolated bacteria were identified as *Vibrio anguillarum*. Only seven samples indicated BKD symptoms while the BKD antigen was present in 43 fish samples (42.3%). The liver of all samples examined showed fatty degeneration and, in addition, 67 samples indicated lipid degeneration of liver. The livers showed necrosis, fatty infiltration and hemorrhage. The fat from liver lesions was classified as lipid deposition as determined by the Nile blue method.

In this study, we classified three causative agents of mortality, namely, vibriosis, BKD and nutritional disease indicated by lipid degeneration of liver.

Tajima et al. (1988) isolated *V. anguillarum* from fish kept in seawater cages. This suggests that the bacteria exists in seawater and that vibriosis occurs when the salmon are stressed.

BKD is a very important disease in coho salmon culture and no treatment exists as yet. Many fish are already infected with this disease in the freshwater environment before transfer to seawater (Sakai et al. 1989). However, the incidence of BKD in fish kept in seawater is greater than that observed in the freshwater environment. Fryer and Sanders (1981) suggested that the symptoms of BKD progressed in fish transferred from freshwater to seawater.

Lipid degeneration of liver in fish has been reported from Atlantic salmon, *Salmo salar*, (Roald 1976) and rainbow trout, *Salmo gairdneri*, (Smith 1979). These reports suggested that lipid degeneration was due to the excessive oxidation of lipids and deficient antioxidant (vitamin E, etc.) in diets. In coho salmon culture, moist pellets containing raw fish (almost all frozen sardines) are used to

feed the fish to provide good growth and food conversion. The lipids of raw fish are susceptible to auto-oxidation or rancidity when exposed to atmospheric oxygen.

It is suggested that the lipid degeneration in coho salmon liver was caused by this nutritionally deficient feed and that death resulted from the cumulative effects of liver disfunction and anemia. Vibriosis, BKD or lipid degeneration did not necessarily appear independently; 3.9% of fish were positive for the three diseases (Table 1). Our results suggest that the fish were stressed by the insufficient

Table 1. The causes of mortalities of pen-cultured coho salmon.

Date	Number of sample	BKD	Vibrio	LD	BKD + Vibrio	BKD + LD	Vibrio + LD	BKD +, Vibrio + LD	Others
April 6	6	3	0	6	0	3	0	0	0
8	6	3	0	4	0	2	0	0	1
9	5	4	1	4	1	3	1	1	0
11	9	7	5	3	3	1	1	0	0
12	5	3	0	1	0	0	0	0	1
22	26	10	1	16	1	6	1	1	6
30	44	13	11	33	2	9	8	2	4
Total	101	43	18	67	7	24	11	4	12
%		42.5	17.8	66.3	6.9	23.7	10.8	3.9	11.8

BKD : Bacterial kidney disease.

Vibrio : Vibriosis.

LD : Lipoid degeneration of the liver.

diet intake which induced one or more of these diseases. Such nutritionally deficient food could be improved by the addition of anti-oxidant (vitamin E, etc.) to the diets of cultured coho salmon. Further study is needed regarding the improvement of salmon diets.

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