Prevalence of *Aeromonas salmonicida*, the causative agent of Furunculosis, among salmonid fish in Japan from 1979-2002.

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Abstract

This study was carried out for the purpose of establishing control methods for furunculosis affecting propagated salmon in Japan. We determined the distribution and prevalence of *A. salmonicida* among asymptomatic adult chum (*Oncorhynchus keta*), pink (*O. gorbuscha*) and masu salmon (*O. masou*) returning to rivers on the island of Hokkaido and on the mainland of Honshu. During 1979-2002, a total of 22,109 chum, pink and masu salmon were collected from the rivers in Hokkaido and Honshu. We isolated *A. salmonicida* from the chum salmon in 21 of the 34 rivers examined and the overall prevalence was 12.2 % in the infected stocks. The prevalence of the agent in pink salmon and masu salmon was 4.6 % and 1.4 % respectively. Using CBB medium, we also examined the chum salmon from the Chitose River catching site. The prevalence of the agent on gill surface was high (50%) at the catching site and decreased among fish in the holding pond in the river. The bacteria appear to be spread during fish migration in the river and during transportation of the fish from the capture site to the holding pond. Recently, the agent was isolated from the gill surface of chum salmon caught off the coast of the eastern part of Hokkaido, but the prevalence was low (1.7%). To control furunculosis in salmonids, the fish should be held for maturation in ponds under conditions of low density and the fish should be disinfected to prevent spread of furunculosis before placement in the holding pond.

Introduction

Furunculosis of salmonid fish was reported firstly in the 1890's by Emmerich et al. (1894). Since these first reports, furunculosis has been reported in virtually all parts of the world where wild or cultured salmonids exist. Serious mortality has been documented in wild adult masu salmon (*Oncorhynchus masou*) and pink salmon (*O. gorbuscha*) in Japan (Kimura, 1970); however, the route of infection for these fish by

the causative agent, *Aeromonas salmonicida*, has not yet been made clear, and such information could assist in the development of more effective control measures for furunculosis. Here, we report the results from an extensive epidemiological study of *A. salmonicida* that was carried out on salmon in Japan.

Material and Methods

Experiment 1: Incidence and distribution of *A. salmonicida* in mature chum, pink and masu salmon showing no apparent clinical signs of furunculosis: From September 1979 to November 2002, a total of 22,109 chum, pink and masu salmon were collected from 35 representative rivers. At each examination, 60 fish were randomly selected from the population. The fish were held in ponds for about one month until maturity, before being processed for examination. Kidney samples were streaked onto nutrient agar plates (Eiken, Tokyo, Japan) and cultured at 20°C for 7 days. No clinical signs of furunculosis were observed in the fish during this portion of the study.

Experiment 2: Incidence of *A. salmonicida* among immature adult chum salmon at a catching site: To better understand the route of infection, we attempted to isolate *A. salmonicida* from kidney, gill and intestine of immature adult chum salmon having no clinical sign of furunculosis were collected in October 2001 and samples were streaked onto CBB medium (Cipriano and Bertolini, 1988). The plates were incubated at 15°C for 5 days.

Experiment 3: Incidence of *A. salmonicida* in chum salmon caught at sea via set net: From 1999-2001, a total of 180 immature adult chum salmon were collected via set net from the Shibetsu coast. Samples of kidney, intestine and gill surface were streaked onto CBB medium.

Results

Experiment 1: Incidence and distribution of *A. salmonicida* in mature chum, pink and masu salmon showing no apparent clinical signs of furunculosis: We isolated *A. salmonicida* from adult chum salmon in 19 of the 32 rivers examined. For pink salmon, 16 rivers were tested and *A. salmonicida* was isolated from fish in 10 of these rivers. From masu salmon, *A. salmonicida* was isolated in 6 of the 10 rivers. In total, we examined 13,281 chum salmon and the incidence of *A. salmonicida* was 12.2%. For pink salmon, 4,305 fish were examined, and the agent was isolated from 4.8% of the fish. In masu salmon, 4,523 fish were examined and the incidence was 1.8%.

A. salmonicida was isolated only from ovarian fluid or from ovarian fluid and kidney samples from fish in the same river. For example, *A. salmonicida* was isolated

from the ovarian fluid of 22 of the 120 fish examined from the Tokachi River. The incidence of *A. salmonicida* in ovarian fluid was not as high as the incidence of the agent in the kidney.

Experiment 2: Incidence of *A. salmonicida* among immature fish at a catching site: In the Ishikari River, no *A. salmonicida* was isolated from the kidney or the intestine of immature chum salmon obtained from the catching site or holding pond. However, *A. salmonicida* was isolated from gill samples of 30 of the 60 adult chum salmon examined (50% incidence) at the catching site in the river. Conversely, in the holding pond, the incidence of *A. salmonicida* from adult gill samples decreased to 20%. The number of *A. salmonicida* organisms present ranged from 10^1 to 10^3 cfu/g gill in the adult chum salmon population from the Ishikari River.

Experiment 3: Incidence of *A. salmonicida* in chum salmon caught at sea via set net: From 1999 to 2001, a total of 180 immature chum salmon were examined from fish collected at the Shibetsu coast. In 2001, the agent was isolated from the gill surface of only one of 60 fish examined. However, the incidence of the agent in mature fish in holding ponds in the area was high in each year that fish were examined.

Discussion

From our data, it appears that the distribution of *A. salmonicida* in salmon from northern Japan can be summarized as follows. A few of the immature adult fish ascending a river have *A. salmonicida* in the kidney, but the incidence increases during maturation to become relatively high. The incidence and level of *A. salmonicida* increases in both the kidneys and the ovarian fluid of mature salmon (Nomura et al. 1992a, Nomura et al. 1992b, Nomura et al. 1993). Ovarian fluid containing *A. salmonicida*, flows out of the fish at the time the eggs are stripped, as well as during the process of final maturation in the pond. Consequently, *A. salmonicida*, together with the ovarian fluid, is expelled into the river water because the effluent from the spawning site and the holding pond is not disinfected in Hokkaido.

We thought from these results that the agent drained from the fish could become an important source of infection for other salmon that ascend the river for spawning. Horne (1928) and McCraw (1952) stated that the most important source of *A. salmonicida* in the spread of furunculosis is the existence of fish carrying this agent. The *A. salmonicida* carrier state in fish poses a serious problem in the prevention of furunculosis and its reduction in fish plays a key role in salmon propagation. To control furunculosis in salmonid fish, the fish should mature in ponds under

conditions of low density and should be disinfected to prevent the spread of furunculosis before placement in the pond for maturation.

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