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Evidence by the Fluorescent Antibody Test for the Occurrence of *Renibacterium salmoninarum* among Salmonid Fish in Chile

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Bacterial kidney disease (BKD) is widespread among hatchery-reared salmonids (Fryer and Sanders, 1981, Ann. Rev. Microbiol. 32: 273–298). The causative agent of BKD, *Renibacterium salmoninarum*, is a fastidious, slow growing, Gram-positive bacillus. The disease is characterized by gray white necrotic abscesses in the kidney. Bacterial kidney disease is now detected routinely in populations of hatchery-reared salmonids in Europe, North America and Japan. During 1983, an epizootic caused by *R. salmoninarum* occurred among chum salmon (*Oncorhynchus keta*) being reared in salt water net pens at Ensenada Baja Hatchery, Chile. After diagnosis, all chum and coho salmon (*Oncorhynchus kisutch*) in adjacent net pens were destroyed. Prior to this epizootic, BKD had not been documented among salmonid stocks in Chile. The purpose of this report is to describe evidence using the direct fluorescent antibody test (DFAT) for the occurrence of *R. salmoninarum* in salmonid stocks of southern Chile.

In May of 1984, kidney tissue was obtained from fish at 10 hatcheries (Fig. 1) and from wild fish collected by gill net at six locations in Chile. Weak, moribund, or freshly dead animals were obtained when available. Smears of kidney tissue made from fish at Bahia Huito and Ensenada Baja Hatcheries during 1983 by Ximena Reyes of the Universidad Católica de Valparaiso were examined also. These tissues were collected from rainbow (*Salmo gairdneri*) and brown (*Salmo trutta*) trout and chinook (*Oncorhynchus tshawytscha*), masu (cherry) (*Oncorhynchus masou*), coho and chum salmon.

Smears of kidney tissue were prepared from each fish. Slides were air-dried, heat-fixed and stored until examined. Each kidney smear was examined using the DFAT for the presence of *R. salmoninarum* (Banner et al., 1982, Bull. Eur. Assoc. Fish Pathol. 2: 35–37; Bullock et al., 1980, Can. J. Fish. Aquat. Sci. 37: 719–721). Individual fish were considered infected when one or more fluorescing bacterial cells with the characteristic size and shape

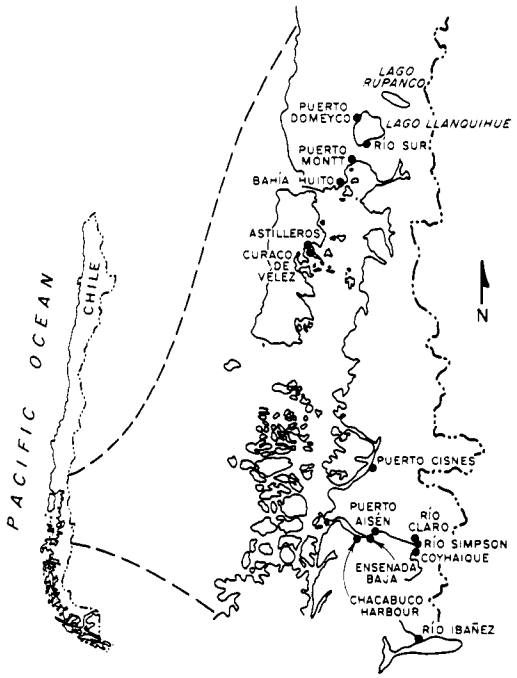

FIGURE 1. Hatcheries (●) in Regions X and XI of Chile from which salmonids were collected.

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of *R. salmoninarum* were observed in smears of kidney tissue.

Salmonid fish with fluorescing bacteria typical of *R. salmoninarum* were found at three hatcheries in Region Ten. This organism was detected in one of 25 fish from one of two lots of coho salmon sampled from salt-water net pens at Bahia Huito Hatchery. Three of 15 dead chinook salmon collected and preserved during 1983 from salt-water net pens at this location also contained these bacteria. At Curaco de Velez Hatchery, samples from the captive chinook salmon brood stock held in salt water and chinook salmon fingerlings from fresh water harbored the organism. No bacterial cells were detected in adult chinook or coho salmon returning from the ocean. At Rio Sur Hatchery, the bacterium was found in coho salmon fingerlings and adults that had returned from Lago Llanquihue to spawn. In both groups only one infected fish was detected. None was detected in chinook salmon from Astilleros or coho salmon from Lago Rupanco and Puerto Dormeyko Hatcheries.

In Region Eleven at Ensenada Baja Hatchery the bacterium was found in coho salmon sampled during 1983 from salt-water net pens next to the chum salmon infected with BKD. The bacterium was also in one of 30 rainbow trout collected from the fresh-water raceway at this location. At Chacabuco Harbour Hatchery the organism was found in one coho salmon from the group in fresh water. None of the coho salmon or brown and rainbow trout examined from the salt-water net pens was infected. One masu salmon from the Rio Simpson Hatchery contained the bacterium, however; none was found among chum and coho salmon examined. Fish from the other two hatcheries in Region Eleven, Río Claro and Puerto Cisnes, and wild rainbow and brown trout from six other locations in this region were not infected.

Clinical signs of the disease were not seen and <2% of the 1,017 fish collected contained fluorescing bacteria with morphology typical of *R. salmoninarum*. These observations are reasonable considering the chronic nature of the BKD and the few mortalities or weak fish examined (Fryer and Sanders, 1981, op. cit.). It is noteworthy that three of the 15 (20%) mortalities collected from Bahia Huito Hatchery and all five coho salmon collected from net pens next to the BKD infected chum salmon at Ensenada Baja Hatchery harbored the bacterium. Routine sampling to detect *R. salmoninarum* often understates the prevalence of this organism in a population. Banner et al. (1983, J. World Maricul. Soc. 14: 236–239) using the DFAT was unable to detect *R. salmoninarum* in any of three 100-fish samples; however after 100 days in pathogen-free salt water about 10% of these animals had died from BKD. These data suggest that, for example, the true prevalence of *R. salmoninarum* in one lot of chinook salmon juveniles from Curaco de Velez Hatchery may be much higher than the 7% prevalence detected by DFAT.

These results suggest the presence of *R. salmoninarum* in salmonid stocks at hatcheries in Chile and are important because they reveal a potential that exists for epizootics of BKD. Salmonid eggs are imported by Chile from Japan and the United States, both areas where BKD is enzootic. Offspring from imported eggs and eggs from adults spawned in Chile were harboring the bacterium. These Chilean stocks are all from imported eggs because native salmonids did not exist in this country. Evidence suggests that *R. salmoninarum* is vertically transmitted and that iodine disinfection does not always prevent transmission of the bacterium (Evelyn et al., 1984, J. Fish Dis. 7: 173–182). *Renibacterium salmoninarum* may have been present in Chile for a number of years, perhaps since the early 1970's when large numbers of salmonid eggs were first imported.

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Survival of *Pasteurella multocida* in Soil and Water in an Area Where Avian Cholera is Enzootic

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Avian cholera has been reported among wildfowl in California since 1944, (Rosen and Bischoff, 1949, Calif. Fish Game 35: 185–192) and in Humboldt County, California since 1945 (Titche, 1979, Calif. Dept. Fish Game, Wildl. Manage. Branch Adm. Rept. 79-2, Sacramento, California, 49 pp.). Despite some studies on the survival of the causative agent, *Pasteurella multocida*, in soil and water (Dimov, 1964, Nauchn. Tr. Vyssh. Vet.-Med. Inst. Sofia 12: 339–345; Olson and Bond, 1968, Proc. Annu. Meet. Livestock Sanit. Assoc. 72: 244–246; Price and Brand, 1984, J. Wildl. Dis. 20: 90–94), the role of soil and water as year-round reservoirs of these bacteria in wildfowl has not been established.

The objectives of this study were to determine 1) whether *Pasteurella multocida* could be isolated from the natural soil or water of an enzootic avian cholera site, and 2) how long detectable concentrations of these bacteria could survive in inoculated soil and water of this site.

The study was conducted at the Centerville Gun Club, a 100 ha area on the Eel River delta of Humboldt County, California. This land is used to pasture sheep and beef cattle between January and October of each year. The waterfowl hunting rights are then leased by eight to 10 local residents from late October to late January.

The ponds lie within 700 m of the Pacific Ocean, from which they are separated by a strip of low, vegetated coastal dunes and some pasturelands. The predominant soil type is a poorly drained soil classified as a siltic clay loam of the Bayside Soil Series (McLaughlin and Harradine, 1965, Soils of Western Humboldt County, California, Dept. Soils and Plant Nutrition, Univ. California, Davis, 85 pp.). To attract waterfowl onto the area, water is pumped from an on-site well to form two shallow ponds, approximately 5.5 ha and 3.9 ha in size, beginning each September. The sizes of these ponds vary each winter with the amount of rainfall.

These two ponds are the only bodies of fresh water in the immediate area and are attractive to migrating waterfowl. They have also been the site of numerous avian cholera epornitics in past years (Titche, 1979, op. cit.; Hazlewood et al., 1978, J. Wildl. Dis. 14: 229–232; Oddo et al., 1978, J. Wildl. Dis. 14: 317–321). In 1977–1978, the winter prior to this study, 1,113 dead wildfowl were observed during an avian cholera epornitic on the study area. Dur-

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