Rickettsial septicemia in farmed Atlantic and chinook salmon in British Columbia: Clinical presentation and experimental transmission

John R. Brocklebank, Trevor P.T. Evelyn, David J. Speare, Robert D. Armstrong

Etiological agents of septicemia most frequently diagnosed in netpen-reared salmon in British Columbia include *Aeromonas salmonicida* (furunculosis) in Atlantic salmon (*Salmo salar*), *Vibrio anguillarum* (vibriosis) in chinook salmon (*Oncorhynchus tshawytscha*), and *Renibacterium salmoninarum* (bacterial kidney disease) in both species. In November 1991, a rickettsia-like organism, similar to an agent described in farmed salmonids in Chile, was suspected as the agent responsible for an outbreak of septicemia resulting in mortality at a growout site located in the coastal waters of Vancouver Island (1). This paper presents the clinical and laboratory findings during investigation of this disease outbreak. In addition, this paper will present brief comments on an experimental transmission study to determine the pathogenicity of the agent.

The affected site consisted of two netpen systems approximately 100 meters apart. The first system consisted of eight cages of 1991-S1, summer, saltwater entry Atlantic salmon, and beyond these cages, there were four cages of 1991-S1, summer, saltwater entry chinook salmon. An "S1" is a smolt or young salmon that has completed the physiological process of smoltification one year after being hatched from the egg. In contrast to an "S1", and "S0" becomes a smolt within a year from being hatched from the egg (2). The second netpen system consisted of eight cages of 1991-S0, spring, saltwater entry chinook salmon. All of the cages were identical, measuring 15 meters square at the surface, and extending to a depth of 20 meters all around. The following values were representative of water conditions at the site at the time of the disease occurrence: temperature was 8°C, salinity was 1.032, and oxygen was 6.0–6.5 mg/L. The site was well-protected and experienced more than adequate tidal movement, so that the cages were regularly flushed free of feed and feces.

Approximately six weeks after an algal (*Heterosigma* sp.) bloom that occurred in September, the daily mortality rate increased steadily during the month of October from 0.01% to 0.06% in two pens of 1991-S1 Atlantic salmon. These two pens were located on opposite sites of a walkway that divided the netpen system down its length. Each pen contained 8500 fish that averaged 400–500 g in weight and came from three different hatcheries. Moribund fish were dark, anorexic, and lethargic. They were located at the surface of the water adjacent to the sides and corners of the cages. On closer examination, all affected fish had similar external lesions; namely, marked bilateral exophthalmia; severe ulcerative stomatitis, extending from the nares to the rostral portion of the ocular sockets; firm, raised, erythematous masses, 1 cm in diameter, that extended from the pseudobranch into the branchial cavity; and solitary shallow ulcers, 1 cm in diameter, located on the flank, craniodorsal to the tail fin.

Internally, there were petechial hemorrhages on the serosal surfaces of the pyloric ceca, swimbladder, and caudal intestine. Fibrous adhesions, attributable to intraperitoneal vaccination with a vaccine containing an oil adjuvant, were found between the spleen, which was mildly enlarged, and the adjacent peritoneum. There was moderate hepatomegaly, with multiple, randomly distributed, 1 cm in diameter, foci of necrosis of the liver capsule (Figure 1); the foci extended from the capsule into the parenchyma to a depth of 0.5 cm, each with a 2 mm wide hyperemic border. On the cut surface, cyst-like spaces, 1 cm in diameter, were present in the parenchyma, but these cysts appeared to be unrelated to the necrotic areas. The etiology of the cysts could not be determined. Although their stomachs were empty, all fish had abundant fat reserves between the pyloric ceca, suggesting that the anorexia had been acute in onset. Fresh dead fish from the two affected pens were necropsied. They had similar external and internal changes to those described in the moribund Atlantic salmon.

Ten healthy fish per pen were arbitrarily sampled with a dip-net from all chinook salmon populations. These fish appeared normal on external and internal examination. No dead fish were available for necropsy. The chinook salmon came from a single hatchery unrelated to the three hatcheries that produced the Atlantic salmon.

The site was revisited in mid-December. Ten healthy fish per pen were arbitrarily selected with a dip-net from all populations of Atlantic and chinook salmon. Internal lesions, similar to those observed in the Atlantic salmon that were sampled in October, were present in seven of the 1991-S0 chinook salmon but not in the 1991-S1 chinook salmon. There were no moribund or fresh dead chinook salmon in the pens. The healthy Atlantic salmon that were sampled appeared normal on necropsy. Moribund Atlantic salmon were only observed lying motionless on the bottom of the seacages and were easily collected. Externally on the moribund Atlantic salmon, there were ulcers and raised, white, firm nodules, 1 cm in diameter, in the skin. The distribution of the nodules was similar to that of the ulcers noted in the Atlantic salmon that were sampled in October.

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EWOS Canada Ltd, Courtenay, British Columbia V9N 2M7 (Brocklebank); Pacific Biological Station, Nanaimo, British Columbia V9R 5K6 (Evelyn); Department of Pathology and Microbiology, Atlantic Veterinary College, University of Prince Edward Island, Charlottetown, Prince Edward Island C1A 4P3 (Speare); Ministry of Agriculture, Fisheries, and Food, Animal Health Centre, Abbotsford, British Columbia V2S 4N8 (Armstrong).

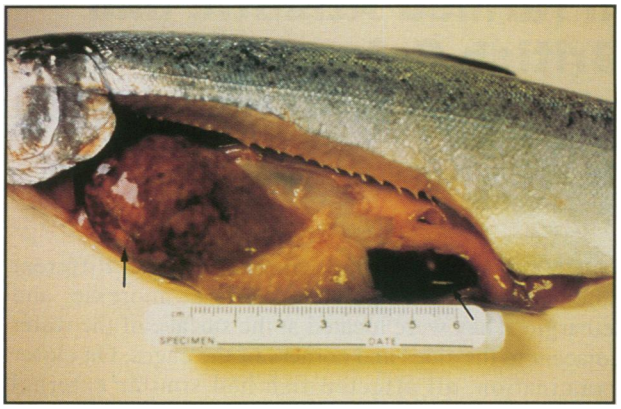

Figure 1. Extensive hepatic changes and splenomegaly in an Atlantic salmon (*Salmo salar*) naturally infected with a rickettsial agent.

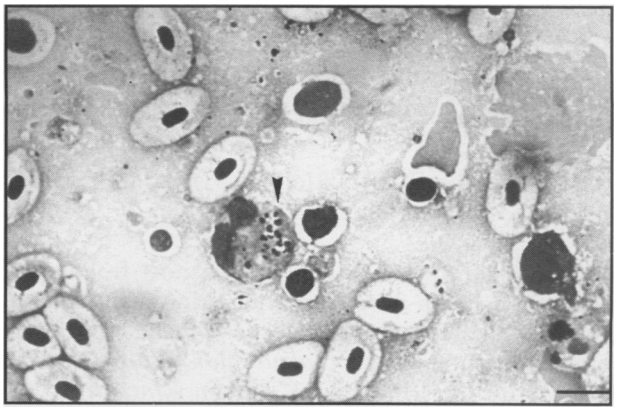

Figure 2. Kidney tissue imprint from an Atlantic salmon naturally infected with a rickettsial agent. Elementary bodies are present within cytoplasmic vacuoles of a macrophage. Gram stain. Bar = 10 μm.

Internal lesions, similar to those already described, were present in the moribund Atlantic salmon. There were no dead Atlantic salmon available for necropsy.

Samples of liver and kidney from the ten moribund Atlantic and the seven affected 1991-S0 chinook salmon were cultured for bacteria on tryptone soy agar. There was no bacterial growth on inoculated agar plates after 10 days incubation at room temperature. Histological examination of formalin-fixed tissue from these same Atlantic and chinook salmon showed a severe chronic necrotizing hepatitis, with fibrosis in advanced lesions and multiple capsular umbilicate foci; also present were severe chronic interstitial nephritis with vasculitis and secondary tubular degeneration, severe chronic necrotizing myocarditis with repair, marked focally extensive pyogranulomatous myositis with ulceration of the overlying epidermis, moderate to marked focally extensive meningoencephalitis, focal pyogranulomatous branchitis, and focal to multifocal subacute pyogranulomatous splenitis with mild multifocal acute vasculitis and parenchymal hemorrhage. Evident in hematoxylin and eosin stained sections of most organs were basophilic spheroid bodies that were consistent in form and size with rickettsia. The bodies were pleomorphic but generally spherical, existing as a coccoid or a diplococcoid with size ranging from 0.5 μm to 1.2 μm in diameter. The bodies were sometimes contained within clear halos. Up to twenty of these bodies could be found in a six-micron thick section of a macrophage (Figure 2). They were gram-negative, acid-fast, and periodic acid-Schiff negative; they stained blue with Macchiavelos and toluidine blue and were positive with Giemsa stain.

Homogenized liver, kidney, and spleen tissue removed from moribund fish was injected intraperitoneally into healthy chinook salmon (40 g) smolts, maintained in sea water at 9°C to 10°C at the Pacific Biological Station. The homogenates were standardized to 2% gram weight of tissue per volume. The homogenates were not standardized to a number of infectious organisms per gram of tissue. Mortalities started at 20–32 days postinoculation and reached 50%–88% by day 50, when the challenge experiments were terminated. Multifocal areas of acute hepatic necrosis were observed in the experimentally infected fish. Intracytoplasmic, membrane-bound vesicles, containing the rickettsia-like organism were seen within macrophages of host tissues of the dead fish (Figure 2). The organism was identified using methylene blue-stained imprints of liver and kidney.

The findings to this point, i.e., clinical presentation, pathology, shape/distribution/staining of the causative agent, and lack of growth on prepared media, suggested a similarity between this outbreak and the so-called Chilean disease of salmonids. To confirm the diagnosis, growth on cell culture and serological tests were attempted. Homogenates (2% g weight/volume) of liver, kidney, and spleen removed from the chinook smolts used in the infectivity study were used to inoculate an epithelioma papillosum cyprini tissue culture maintained at 15°C. The tissue culture did not contain antibiotics. Resulting cytopathic effects observed after four weeks of incubation included focal rounding-up of cells and focal, followed by a generalized, exfoliation of cell layers. An indirect fluorescent antibody test (IFAT) (J.L. Fryer, Oregon State University, Corvallis, Oregon) was applied to infected liver, kidney, and spleen smears prepared from the chinook smolts used in the infectivity study, and to the infected tissue culture. The IFAT detected a rickettsial agent in these tissues.

Homogenates (2% g weight/volume) from moribund chinook salmon liver tissue that were infected with the rickettsial organism were no longer capable of inducing the disease or causing cytopathological changes in chinook smolts if they were incubated at 37°C for 16 hours prior to injection into the chinook smolts.

All fish at the growout site were given oxytetracycline per os (Terramycin Aqua, Pfizer Canada Ltd., Calgary, Alberta), mixed into the feed at 100 mg/kg fish for 21 days, as tissue culture studies had suggested some sensitivity of the organism to oxytetracycline (3), and oxytetracycline is registered for use in food fish in Canada. One week after the last day of treatment, the mortality rate had fallen from 0.66% to 0.015% per day in the Atlantic salmon, but then it gradually rose to 0.02% per day after the fourth week and remained at this level for an additional ten weeks until mid-May. It could not be determined whether the reduction in mortality rate was in response to the treatment or due to the development of host immunity. In Chile, no particular treatment regime has been successful in preventing or controlling this infection (4). Good husbandry prac-

tices, such as maintaining separate year classes and single species at each site, may be the only practical measures to reduce the prevalence of the infection in both Chile and British Columbia (5).

The history, clinical signs, postmortem findings, growth on cell culture, and IFAT results strongly suggest that the disease at this growout site was caused by a rickettsial agent similar to the one affecting farmed coho salmon in Region X of Chile (3). The disease has been called "Chilean disease" (5), "coho salmon syndrome" (3), "Huito disease" (3), and, perhaps more appropriately, "salmonid rickettsial septicemia" (3). Fryer *et al* (6) named the causative agent, *Piscirickettsia salmonis*; it is an obligate intracellular parasite that multiplies only within living host cells (3,5).

The Chilean epizootics typically began after periods of environmental stress (fluctuating sea temperatures, nontoxic algal blooms, severe storms) in the Chilean equivalent of autumn to mid-winter (3,4). The seasonal occurrence and temporal association with environmental stress that were seen in Chile are similar to what we observed in British Columbia. Branson and Nieto Diaz-Munoz (4) have suggested that severe stress or inadequate nutrition may be a necessary precipitating factor for the disease in Chile.

In Chile, severe losses in coho salmon with cumulative mortalities of more than 90% on some sites occurred during the first year, 10–12 weeks after seawater introduction (4). The monthly mortality rate varied between 1% and 20%, and rose to 40% during epizootics (3). The Chilean epizootics sometimes lasted for 10 weeks before subsiding (3). Repeat outbreaks occurred from October through December (spring to midsummer in Chile) (3). At the affected culture site in British Columbia, the cumulative mortality was 8% for the Atlantic salmon and negligible for the chinook salmon. Repeat outbreaks did not occur.

As in the Chilean situation, the most consistent finding associated with the disease was the randomly distributed ring-shaped lesion seen in the capsule and parenchyma of the liver (3,5). In the acute stage of the rickettsial infection in Chile, affected fish had enlarged kidneys and spleen, and pale gills due to severe anemia (Sandra Bravo, personal communication). These clinical signs were not observed in either the naturally or the experimentally affected salmon in British Columbia.

Salmonid diseases in British Columbia due to rickettsia and chlamydia are rare (5). Rickettsial septicemia in salmon in British Columbia is not listed in the Fish Health Protection Regulations (7). A similar, probably identical, disease, "parenthesis disease", has been recognized since 1970 in British Columbia in salmon held in seawater (5). It was first observed in 1970 and 1978 in pink salmon (*Oncorhynchus gorbuscha*) that were being cultured in seawater tanks for experimental purposes at the Pacific Biological Station, and later in 1983 and 1984, in farmed coho and chinook salmon (5). While the condition was always fatal for pink salmon, its occurrence in the farmed chinook and coho salmon was always coincidental to some other more serious problem (5).

Studies on the outbreak in pink salmon in 1970 demonstrated that the infection could be transmitted not only by intraperitoneal injection of homogenized infected tissue from pink salmon, but also by cohabitation with infected pink salmon. Injection of affected tissue was lethal for pink, chinook, and coho salmon. Similar results have been obtained by Garces *et al* (8) for Atlantic and coho salmon, and by ourselves for chinook salmon.

Rickettsiae have been horizontally transmitted in seawater to Atlantic and chinook salmon and rainbow trout (*Oncorhynchus mykiss*) (Walbaum) without a marine arthropod (sea lice) vector (3,4). Sea lice (such as *Lepeophtheirus salmonis* or *Caligus clemensi*) were not observed on the Atlantic or chinook salmon from British Columbia prior to, or on, either sampling date. These findings suggest that a vector is not required for the transmission of rickettsia to fish. Rickettsial infection of fish appears to differ from the rickettsial infection of dogs (ehrlichiosis and Rocky Mountain spotted fever), which are tick-transmitted diseases, and salmon poisoning of dogs, which results from the ingestion of fish parasitized with a rickettsia-carrying digenetic trematode (9). Rickettsial infections of fish and mammals are similar, since the infective agent is spread via the blood to vascular endothelial cells of capillary beds (4,9). The result is an acute systemic vasculitis with thrombosis of the vascular lumen (4). These vascular lesions may lead to focal necrosis (4) and, as a consequence, to the skin lesions observed in the Atlantic salmon from British Columbia (4).

Rickettsiae have been found in the ovary and testes of affected salmon in Chile (3) and British Columbia (1). However, they have never been observed in the freshwater rearing phase of salmon, and thus vertical transmission appears unlikely (3,5). To prevent the introduction of rickettsiae into Chilean spawning facilities, fish tissues are routinely screened by Gram's stain, and eggs are sanitized with iodophores (3). These same preventive measures are already in place in most hatcheries in British Columbia to control furunculosis and bacterial kidney disease.

The origin of the rickettsial organism that affected farmed salmon in British Columbia has not been determined. Branson and Nieto Diaz-Munoz (4) have suggested that it may have originated from a local marine source, because the affected fish in Chile and British Columbia came from different hatcheries and had been in the sea for several weeks prior to contracting the condition. Reservoirs of rickettsia may exist in transient and resident nonsalmonid fish that move through netpens or in marine shellfish (3). Rickettsial infections have been observed in Pacific coast molluscs and crustacea (10). How long and under what conditions rickettsiae can survive in seawater is not known (3).

Acknowledgments

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Correction—Can Vet J 1993; 34: 686-688

Repair of fractures of the radius and ulna in a ewe using positive profile transfixation pins and casting

David E. Anderson, Guy St.Jean

In the November issue of the *CVJ*, figures 2 and 3 of the above mentioned article were accidentally transposed. We apologize for this error. The photos are reprinted correctly below. Eds.

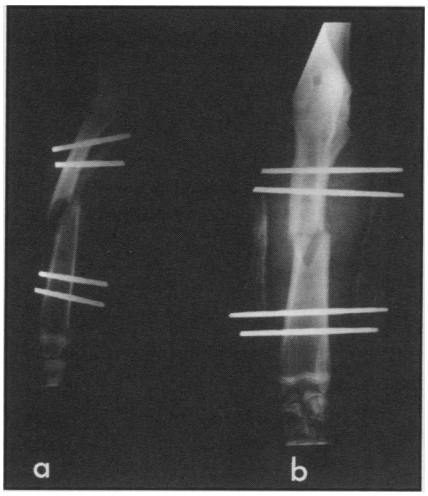

Figure 2. Lateral (a) and craniocaudal (b) views of left forelimb after transfixation pinning and casting for a radial-ulnar fracture. Note the use of centrally threaded, positive profile transcortical pins.

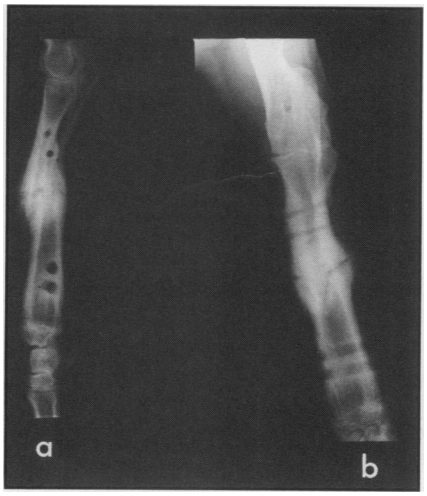

Figure 3. Lateral (a) and craniocaudal (b) views of left forelimb after transfixation pin and cast removal eight weeks after surgery. A bridging callus is seen incorporating the radius and ulna. The fracture line is still apparent.