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SHORT COMMUNICATION

First isolation of *Piscirickettsia salmonis* from coho salmon, *Oncorhynchus kisutch* (Walbaum), and rainbow trout, *Oncorhynchus mykiss* (Walbaum), during the freshwater stage of their life cycle

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Since 1989, a significant number of mariculture facilities in southern Chile have been affected by extensive mortality among salmonid fish reared in salt water. Losses have been as high as 90% at some affected farms (Bravo & Campos 1989). Fryer, Lannan, Garcés, Larenas & Smith (1990) isolated the aetiologic agent of the disease by directly inoculating infected fish tissue onto monolayers of CHSE-214 cells (ATCC CRL 1681) (Lannan, Winton & Fryer 1984). This agent was later classified as a member of the order Rickettsiales, family Rickettsiaceae (Fryer, Lannan, Giovannoni & Wood 1992). This obligatory intracellular bacterium, the first of its kind isolated from fish, has been named *Piscirickettsia salmonis* and the type strain has been designated LF-89 (ATCC VR 1361). Similar infections have been reported in Canada, Ireland and Norway (Rodger & Drinan 1993; Lannan & Fryer 1993; Olsen, Evensen, Speilberg, Melby & Hastein 1993).

Piscirickettsia salmonis can be grown in salmonid cell lines, including CHSE-214, in an antibiotic-free medium at 15–18 °C (Fryer *et al.* 1990). The characteristic cytopathic effect (CPE) appears in the monolayer 5–10 days after inoculation and consists of clusters of rounded cells. *Piscirickettsia salmonis* is a Gram (−ve) pleomorphic bacterium with a diameter of about 0·5–1·5 μm. It is predominantly coccoid, but it can also occur as rings or pairs of curved rods (Fryer *et al.* 1990).

The clinical signs and gross pathology associated with the disease include lethargy and darkening of infected fish, with white spots and ulcerations of the skin. The gills appear pale, and the abdomen distended and filled with ascites. The kidney is swollen, the spleen enlarged and grey mottled lesions are occasionally presented on the liver (Branson & Nieto Diaz-Muñoz 1991).

The disease was originally thought to be confined to coho salmon, *Oncorhynchus kisutch* (Walbaum). However, it is now known to affect chinook salmon, *Oncorhynchus tshawytscha* (Walbaum), Atlantic salmon, *Salmo salar* L., and rainbow trout, *Oncorhynchus mykiss* (Walbaum). The disease has not been thought to affect fish during freshwater rearing and mortality normally begins 6–12 weeks after fish are introduced into sea water (Fryer *et al.* 1992). Although Bravo (1994) first reported the occurrence of *P. salmonis* in rainbow trout in

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fresh water, in this paper we record the first isolation of *P. salmonis* from diseased fish during the freshwater stage of their life cycle.

The study fish, coho salmon, Atlantic salmon and rainbow trout, were obtained during a natural epizootic in September 1993, from several freshwater fish farms located in Chiloé Island, $\bar{X}$ region, Chile. The diseased fish were 60–90 days old and about 8–10 cm in length. They were very dark in colour with white round spots on the skin. However, none showed skin ulcerations, as observed in diseased fish from salt water. There was some abdominal distension and ascites. The gills were pale, as were the liver and kidney.

Kidney tissue from affected fish was aseptically removed, homogenized and inoculated on CHSE-214 cell monolayers as described previously (Fryer *et al.* 1990). The first signs of a CPE, which consisted of the formation of cell clusters in some areas of the monolayer, appeared in the coho salmon and rainbow trout kidney inoculated CHSE-214 monolayers about 5 days post-inoculation. The CPE occupied the whole monolayer around the fourteenth day of incubation. The observed CPE in CHSE-214 cells was similar to that described for *P. salmonis* in these cells (Fryer *et al.* 1990). In a parallel experiment, monolayers of CHSE-214 cells were inoculated with homogenized kidney tissue, but in the presence of antibiotics (penicillin 100 IU ml^{-1}, streptomycin 100 $\mu g\,ml^{-1}$ and fungizone 2·5 $\mu g\,ml^{-1}$). No CPE was observed in these cultures. Moreover, no bacterial growth was seen in media [trypticase soy agar (TSA) and kidney disease medium (KDM)] routinely used to culture fish-pathogenic bacteria.

Smears made from a pellet of centrifuged supernatants from CPE-positive inoculated cell cultures were stained with Gram and Giemsa stains. Gram staining (Fig. 1) showed differing

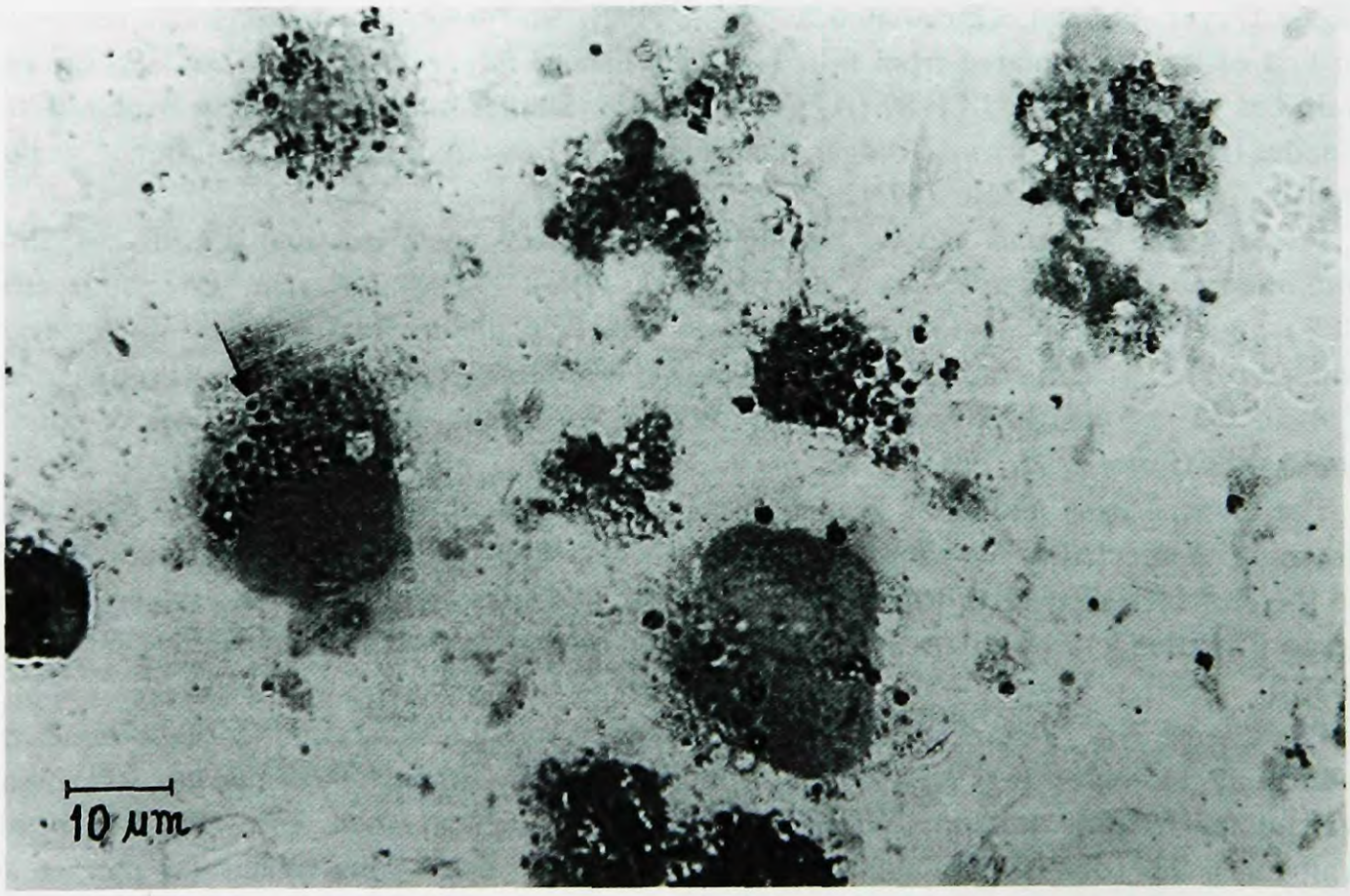

Figure 1. Smear of a pellet from centrifuged supernatants from CPE-positive inoculated CHSE-214 cells. Cells show a large number of *Piscirickettsia salmonis* organisms in their cytoplasm. Note extensive cellular destruction and large numbers of organisms within an intracytoplasmic vacuole (arrow) (Gram stain, ×1000).

sizes of Gram-ve pleomorphic cocci in pairs or forming groups within cytoplasmatic vacuoles. Giemsa staining showed the same coccoid organisms. Furthermore, the smears were submitted to a *P. salmonis* IFAT assay (Lannan, Ewing & Fryer 1991). The IFAT assay was positive for the agent tested. These results confirm the presence of *P. salmonis* in the assayed fish.

The origin of the *P. salmonis* infection in these fish is not certain. The pathogen may have been vertically transmitted, since the broodstock from which the fish were derived were the survivors of a saltwater *P. salmonis* outbreak. Alternatively, it may be of freshwater origin. The stress of transfer between sites may have led to the outbreak of disease.

Affected fish also had a concurrent *Renibacterium salmoninarum* infection which may have influenced the appearance of clinical *P. salmonis* disease. *Renibacterium salmoninarum* was isolated from the fish after the appearance of *P. salmonis*. A 2–3 °C rise in water temperature was observed in the affected farms before mortalities occurred. A rise of water temperature has also been thought to trigger the appearance of disease in salt water (Branson & Nieto Díaz-Muñoz 1991).

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