

## 1.2.13 Piscirickettsiosis

**Marcia L. House and John L. Fryer**

Center for Salmon Disease Research  
Department of Microbiology  
Oregon State University  
Corvallis, OR 97331-3804

[mhouse@nwifc.org](mailto:mhouse@nwifc.org)

[fryerj@bcc.orst.edu](mailto:fryerj@bcc.orst.edu)

### A. Name of Disease and Etiological Agent

#### 1. Name of Disease

Piscirickettsiosis, salmonid rickettsial septicemia, coho salmon septicemia, and Huito disease.

#### 2. Etiological Agent

*Piscirickettsia salmonis*

This organism is an intracellular rickettsial-like pathogen of fish that replicates within membrane-bound cytoplasmic vacuoles of infected cells. The bacterium is fastidious and does not grow on any known artificial media. It is distantly related to the genera *Coxiella* and *Francisella*, and is grouped with the gamma subdivision of the proteobacteria. J. L. Fryer and C. N. Lannan have suggested this bacterium be included in a new family Piscirickettsiaceae and the proposal for the family with the formal description will appear in volume 2, second edition of Bergey's Manual of Systematic Bacteriology (expected publication date in 2002).

### B. Known Geographical Range and Host Species of the Disease

#### 1. Geographical Range

Isolated from salmonid fish in Chile, Ireland, Norway, and both the west and east coasts of Canada.

#### 2. Host Species

Piscirickettsiosis has been observed in or isolated from coho salmon (*Oncorhynchus kisutch*), chinook salmon (*O. tshawytscha*), sakura salmon (*O. masou*), rainbow trout (*O. mykiss*), pink salmon (*O. gorbuscha*), and Atlantic salmon (*Salmo salar*). Coho salmon appear most susceptible. Other species of fish may also be susceptible to this bacterium.

### C. Epizootiology

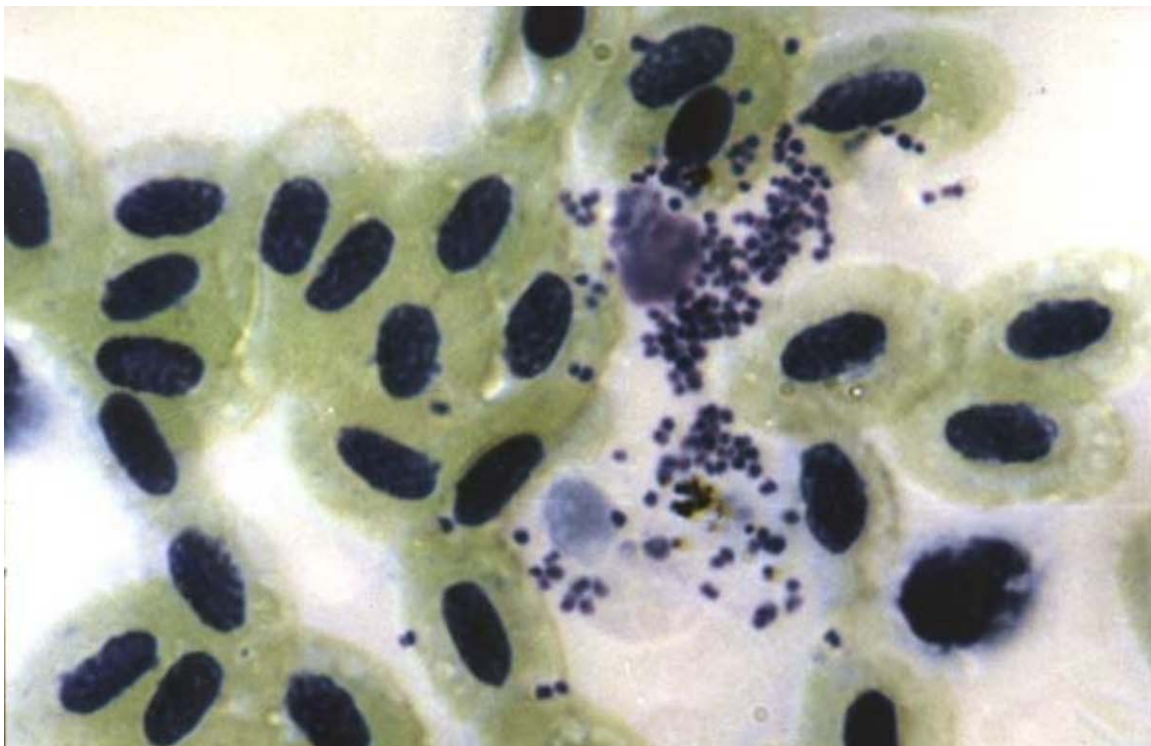
Piscirickettsiosis was initially described in 1989 from infected salmonids in Chile. Onset of mortality occurred 6 to 12 weeks after farmed coho salmon had been moved to seawater netpens. Since that time, the disease has been primarily reported in farmed marine fish and has also been observed in salmonids from freshwater facilities. The mechanisms of transmission are not completely understood. Horizontal transmission in salt and freshwater has been demonstrated. Transmission by vectors and the role of vertical transmission remains uncertain. Different isolates have been shown to have varying levels of virulence.

### D. Disease Signs

The first evidence of disease may be the appearance of small white lesions or shallow haemorrhagic ulcers on the skin. Affected fish appear dark, lethargic, and collect along the sides of the netpen. The major gross pathological changes are gill pallor, peritonitis, ascites, enlarged spleen, swollen grey kidney, and liver with large pale necrotic lesions.

### E. Disease Diagnostic Procedures

Piscirickettsiosis is diagnosed based on clinical signs, isolation of *P. salmonis* in cell culture, and detection in Giemsa stained tissue impressions or sections. It is pleomorphic, ranging from 0.5 to 1.5  $\mu\text{m}$  in diameter, and frequently occurs in pairs (Figure 1). Positive identification is confirmed using *P. salmonis*-specific antibodies in the IFAT.

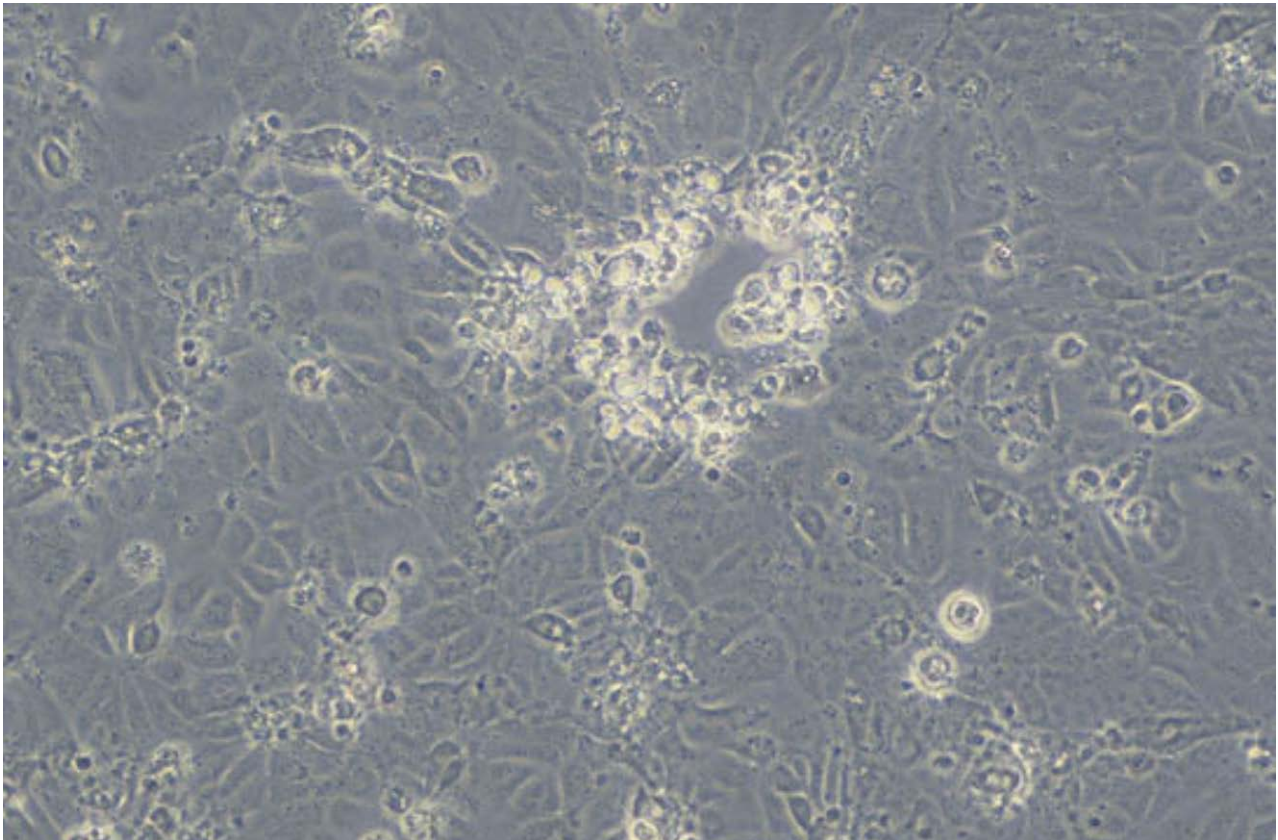


**Figure 1.** Blood smear from coho salmon infected with *Piscirickettsia salmonis*.

### 1. Presumptive Diagnosis

*Piscirickettsia salmonis* is a gram-negative, non-motile, highly fastidious intracellular bacterial pathogen. The organism does not grow on artificial bacteriological media and fish cell cultures are required for isolation. The techniques used to isolate and culture *P. salmonis* are more similar to those used in virology than traditional bacteriology. **DUE TO SENSITIVITY OF *P. SALMONIS* TO ANTIBIOTICS *IN VITRO*, NONE SHOULD BE USED IN MEDIA DURING COLLECTION OF TISSUE OR THE CULTURE OF CELLS.**

Samples of kidney, liver, and blood suitable for bacteriological testing are aseptically collected from diseased fish during either overt or covert infections as described in Section 1, 1.1.1 General Procedures for Bacteriology. Tissue should be homogenized at 1/20 in antibiotic-free balanced salt solution (BSS), and then, **WITHOUT CENTRIFUGATION**, further diluted 1/5 and 1/50 in antibiotic free BSS for inoculation onto cell cultures. *Piscirickettsia salmonis* cells are bound in membranes and centrifugation will remove the bacteria from the supernatant. Final dilutions for use are  $10^{-2}$  and  $10^{-3}$ . The diluted homogenate can be inoculated directly (0.1 mL/culture) into the antibiotic-free culture medium overlaying the CHSE-214 cell monolayer. The cell cultures must be incubated at 15 to 18°C for 28 days and observed for the appearance of cytopathic effect (CPE). The piscirickettsial CPE consists of plaque-like clusters or rounded cells (Figure 2). With time, the CPE progresses until the entire cell sheet is destroyed. If CPE does not occur (except in the positive controls), cultures should be incubated at 15 to 18°C for an additional 14 days.



**Figure 2.** Cytopathic effect (CPE) of *Piscirickettsia salmonis* grown in a CHSE-214 cell culture.

## 2. Confirmatory Diagnosis

Positive identification using *P. salmonis*-specific antibodies in the IFAT.

## F. Procedures for Detecting Subclinical Infections

No procedures have been reported.

## G. Procedures for Determining Prior Exposure to the Etiological Agent

No procedures have been reported.

## H. Procedures for Transportation and Storage of Samples to Ensure Maximum Viability and Survival of the Etiological Agent

**DO NOT USE ANTIBIOTICS IN THE ISOLATION PROCEDURE.** For isolation, the tissue, preferably the kidney, must be aseptically removed and transferred to a sterile container, and, if possible, prepared immediately. If it is not possible to process tissues immediately, they must be kept at 4°C or on ice. **DO NOT FREEZE.** Further preparation should take place as soon as possible (within 6 to 12 hours), as the risk of overgrowth by contaminants increases with time. Smears or impressions of the kidney, liver, and spleen should be prepared, air-dried, and fixed for five minutes in absolute methanol. Tissues smears to be examined by IFAT must be freshly prepared or stored at  $\leq -20^{\circ}\text{C}$ .

## References

- Alday-Sanz, V., H. Rodger, T. Turnbull, A. Adams, and R. H. Richards. 1994. An immunohistochemical diagnostic test for rickettsial disease. *Journal of Fish Diseases* 17:189-191.
- Almendras, F. E., I. C. Fuentealba, S. R. M. Jones, F. Markham, and E. Spangler. 1997. Experimental infection and horizontal transmission of *Piscirickettsia salmonis* in freshwater-raised Atlantic salmon, *Salmo salar*. *Journal of Fish Diseases* 20:409-418.
- Bravo, S. 1994. Piscirickettsiosis in freshwater. *Bulletin of the European Association of Fish Pathologists* 14(4):137-138.
- Bravo, S., and M. Campos. 1989. Coho salmon syndrome in Chile. *American Fisheries Society/Fish Health Section Newsletter* 17(3):3.
- Brocklebank, J. R., D. J. Speare, R. D. Armstrong, and T. Evelyn. 1992. Septicemia suspected to be caused by a rickettsia-like agent in farmed Atlantic salmon. *Canadian Veterinary Journal* 33:407-408.
- Cvitanich, J. D., N. O. Garate, and C. E. Smith. 1991. The isolation of a rickettsia-like organism causing disease and mortality in Chilean salmonids and its confirmation by Koch's postulate. *Journal of Fish Diseases* 14:121-145.

- Evelyn, T. P. T. 1992. Salmonid Rickettsial Septicemia. Pages 18-19 in M. L. Kent, editor. Diseases of Seawater Netpen-reared Salmonid Fishes in the Pacific Northwest. Canadian Special Publication Fish Aquatic Science 116. Department of Fisheries and Oceans, Nanaimo, B. C.
- Fryer, J. L., C. N. Lannan, L. H. Garcès, J. J. Larenas, and P. A. Smith. 1990. Isolation of a rickettsiales-like organism from diseased coho salmon *Oncorhynchus kisutch* in Chile. *Fish Pathology* 25(2):107-114.
- Fryer, J. L., C. N. Lannan, S. J. Giovannoni, and N. D. Wood. 1992. *Piscirickettsia salmonis* gen. nov., sp. nov., the causative agent of an epizootic disease in salmonid fishes. *International Journal of Systematic Bacteriology* 42(1):120-126.
- Gaggero, A., H. Castro, and A. M. Sandino. 1995. 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. *Journal of Fish Diseases* 18:277-279.
- Garcès, L. H., J. J. Larenas, P. A. Smith, S. Sandino, C. N. Lannan, and J. L. Fryer. 1991. Infectivity of a rickettsia isolated from coho salmon (*Oncorhynchus kisutch*). *Diseases of Aquatic Organisms* 11:93-97.
- House, M. L., J. L. Bartholomew, J. R. Winton, and J. L. Fryer. 1999. Relative virulence of three isolates of *Piscirickettsia salmonis* for coho salmon *Oncorhynchus kisutch*. *Diseases of Aquatic Organisms* 35:107-113.
- Jones, S. R. M., R. J. F. Markham, D. B. Groman, and R. R. Cusack. 1998. Virulence and antigenic characteristics of a cultured Rickettsiales-like organism isolated from farmed Atlantic salmon *Salmo salar* in Eastern Canada. *Diseases of Aquatic Organisms* 33:25-31.
- Lannan, C. N., and J. L. Fryer. 1991. Recommended methods for inspection of fish for the salmonid rickettsia. *Bulletin of the European Association of Fish Pathologists* 11:135-136.
- Lannan, C. N., S. A. Ewing, and J. L. Fryer. 1991. A fluorescent antibody test for detection of the rickettsia causing disease in Chilean salmonids. *Journal of Aquatic Animal Health* 3:229-234.
- Mael, M.J., S. J. Giovannoni, and J. L. Fryer. 1996. Development of polymerase chain reaction assays for detection, identification, and differentiation of *Piscirickettsia salmonis*. *Diseases of Aquatic Organisms* 26:189-195.
- Mael, M. J., S. J. Giovannoni, and J. L. Fryer. 1999. Phylogenetic analysis of *Piscirickettsia salmonis* by 16S, internal transcribed spacer (ITS) and 23S ribosomal DNA sequencing. *Diseases of Aquatic Organisms* 35:115-123.
- Olsen, A. B., H. P. Melby, L. Speilberg, O. Evensen, and T. Håstein. 1997. *Piscirickettsia salmonis* infection in Atlantic salmon *Salmo salar* in Norway - epidemiological, pathological, and microbiological findings. *Diseases of Aquatic Organisms* 31(1):35-48.
- Palmer, R., M. Ruttledge, K. Callanan, and E. Drinan. 1997. A Piscirickettsiosis-like disease in farmed Atlantic salmon in Ireland - isolation of the agent. *Bulletin of the European Association of Fish Pathologists* 17(2) 68-72.

### 1.2.13 Piscirickettsiosis - 6

Rodger, H. D., and E. M. Drinan. 1993. Observation of a rickettsia-like organism in Atlantic salmon, *Salmo salar* L., in Ireland. *Journal of Fish Diseases* 16:36