Vol. 35: 107–113, 1999 | DISEASES OF AQUATIC ORGANISMS Dis Aquat Org | Published January 29

Relative virulence of three isolates of *Piscirickettsia salmonis* for coho salmon *Oncorhynchus kisutch*

M. L. House[1,2], J. L. Bartholomew[1], J. R. Winton[2], J. L. Fryer[1,*]

[1]Center for Salmon Disease Research and Department of Microbiology, Oregon State University, Corvallis, Oregon 97331-3804, USA

[2]Western Fisheries Research Center, United States Geological Survey, 6505 NE 65th St., Seattle, Washington 98115, USA

ABSTRACT: *Piscirickettsia salmonis* was first recognized as the cause of mortality among pen-reared coho salmon *Oncorhynchus kisutch* in Chile. Since the initial isolation of this intracellular Gram-negative bacterium in 1989, similar organisms have been described from several areas of the world, but the associated outbreaks were not reported to be as serious as those that occurred in Chile. To determine if this was due to differences in virulence among isolates of *P. salmonis*, we conducted an experiment comparing isolates from Chile, British Columbia, Canada, and Norway (LF-89, ATL-4-91 and NOR-92, respectively). For each of the isolates, 3 replicates of 30 coho salmon were injected intraperitoneally with each of 3 concentrations of the bacterium. Negative control fish were injected with MEM-10. Mortalities were collected daily for 41 d post-injection. Piscirickettsiosis was observed in fish injected with each of the 3 isolates, and for each isolate, cumulative mortality was directly related to the concentration of bacterial cells administered. The LF-89 isolate was the most virulent, with losses reaching 97% in the 3 replicates injected with $10^{5.0}$ $TCID_{50}$, 91% in the replicates injected with $10^{4.0}$ $TCID_{50}$, and 57% in the fish injected with $10^{3.0}$ $TCID_{50}$. The ATL-4-91 isolate caused losses of 92% in the 3 replicates injected with $10^{5.0}$ $TCID_{50}$, 76% in the fish injected with $10^{4.0}$ $TCID_{50}$, and 32% in those injected with $10^{3.0}$ $TCID_{50}$. The NOR-92 isolate was the least virulent, causing 41% mortality in the replicates injected with $10^{4.6}$ $TCID_{50}$. At 41 d post-injection, 6% of the fish injected with $10^{3.6}$ $TCID_{50}$ NOR-92 had died. Mortality was only 2% in the fish injected with $10^{2.6}$ $TCID_{50}$ NOR-92, which was the same as the negative control group. Because the group injected with the highest concentration ($10^{4.6}$ $TCID_{50}$) of NOR-92 was still experiencing mortality at 41 d, it was held for an additional 46 d. At 87 d post-injection, the cumulative mortality in this group had reached 70%. These differences in virulence among the isolates were statistically significant ($p < 0.0001$), and are important for the management of affected stocks of fish.

KEY WORDS: *Piscirickettsia* · Virulence · Salmon · Disease

INTRODUCTION

In 1989, a novel bacterial fish pathogen, *Piscirickettsia salmonis*, LF-89^T (Fryer et al. 1992), was isolated from netpen-cultured coho salmon *Oncorhynchus kisutch* during an epizootic in Region 10, Chile (Fryer et al. 1990). Losses approaching 90% were reported (Bravo & Campos 1989), with the onset of mortality occurring 6 to 12 wk after the fish were transferred from freshwater to marine netpens. Moribund fish were lethargic, dark in color, and swam at the edges of the pens near the surface. Gross pathology included swollen kidneys and enlarged spleens. Other characteristics seen in some, but not all, of the affected fish were anemia (hematocrit 27% or less), ascites, mottled livers with grey circular lesions, and petechial hemorrhages in the stomach, intestines, pyloric caeca, and visceral fat. Completion of Koch's postulates identified *P. salmonis* as the causative agent of the epizootics, and the disease was named piscirickettsiosis (Cvitanich et al. 1991, Fryer et al. 1992).

Following the initial characterization of the Chilean LF-89 strain of *Piscirickettsia salmonis* (Fryer et al.

*Addressee for correspondence. E-mail: fryerj@bcc.orst.edu

1992), similar Gram-negative intracellular bacterial pathogens of salmonids were described from other areas of the world. In British Columbia, Canada, *P. salmonis* was isolated from netpen-cultured Atlantic (*Salmo salar*) and chinook (*Oncorhynchus tshawytscha*) salmon (Brocklebank et al. 1993). In 1992, *P. salmonis* was recovered from Atlantic salmon cultured in seawater netpens on the west coast of Norway (Olsen et al. 1997). Researchers in Ireland have observed (Rodger & Drinan 1993) and recently isolated from netpen-cultured Atlantic salmon (Palmer et al. 1997) a Gram-negative intracellular pathogen that was identified as *P. salmonis* using an immunohistochemical test with polyclonal antiserum (Alday-Sanz et al. 1994). Additionally, *P. salmonis* was isolated from netpen-cultured Atlantic salmon in Nova Scotia, Canada (Cusack et al. 1997, Jones et al. 1998). None of these isolates was reported to be as virulent as the initial Chilean isolate.

Sequence comparison of the 16S ribosomal RNA genes of *Piscirickettsia salmonis* isolates from Chile, British Columbia, Canada, and Norway (Mauel 1996) demonstrated that these rickettsia-like organisms were closely related. The genetic analysis offered no explanation for the observed differences in losses from piscirickettsiosis in different parts of the world. Factors that could contribute to the reported variation include differences in the virulence of the isolates, host susceptibility, environmental conditions, or co-infections with other pathogens. The purpose of this study was to determine if geographically distinct isolates of *P. salmonis* differed in virulence for coho salmon.

MATERIALS AND METHODS

***Piscirickettsia salmonis* isolates.** Three isolates of *P. salmonis* were used in this study. The type strain, LF-89, was isolated from the kidney of a diseased 2-year-old coho salmon from a seawater netpen site in Southern Chile (Fryer et al. 1990, 1992). The isolate from British Columbia, ATL-4-91, was obtained from netpen-raised Atlantic salmon and was provided by Dr G. Traxler, Department of Fisheries and Oceans, Nanaimo, Canada. An isolate from netpen-raised Atlantic salmon from Norway, NOR-92, was provided by Dr H. P. Melby, National Veterinary Institute, Oslo, Norway. The bacteria were grown at 15°C in CHSE-214 cell cultures (Lannan et al. 1984) using antibiotic-free Eagle's minimum essential medium (Automod, Sigma Chemical Co., St. Louis, MO) supplemented with 10% fetal bovine serum (MEM-10, Hyclone Laboratories, Inc., Logan, UT) as described by Fryer et al. (1990). Each isolate was passed 5 times in CHSE-214 cells, for a total of 9 passes in cell culture since isolation. Bacterial cells were harvested at 10 to 11 d post-inoculation when the monolayer showed complete cytopathic effect (CPE). The rate of development of CPE appeared similar for all isolates. An endpoint dilution assay ($TCID_{50}$) was used to determine the infectivity titer of the inocula. This assay used CHSE-214 cells in a 96-well tissue culture plate and was initiated the same day that fish were inoculated. The 96-well plates in the endpoint dilution assay were incubated at 15°C for 28 d, then visually inspected for the presence of CPE. Dilution endpoints were determined by the method of Reed & Muench (1938).

Experimental salmon. Approximately 1000 coho salmon (avg. weight 12 g) were obtained from the Oregon Department of Fish & Wildlife (ODF&W), Cascade Locks Hatchery, USA. Previous health examinations did not detect the presence of bacterial or viral fish pathogens. The fish were transported to holding facilities at the Western Fisheries Research Center in Seattle, Washington, USA, and maintained in aquaria provided with 12°C, sand-filtered, UV-treated freshwater from Lake Washington. At the initiation of the experiment, fish were moved into the Biosafety Level 3 (BL-3) containment facility.

Experimental design. Relative virulence of the 3 isolates was determined by comparing the cumulative mortalities and survival times among 3 treatment levels for each isolate. Each treatment and a control group consisted of 3 replicate tanks of 30 fish each. Thus, 90 fish were injected intraperitoneally with 0.1 ml of each of 3 concentrations of each isolate of *Piscirickettsia salmonis* suspended in MEM-10. Fish in the control group received 0.1 ml of MEM-10. Endpoint dilution assays indicated that isolates LF-89 and ATL-4-91 were delivered at doses of $10^{5.0}$, $10^{4.0}$, or $10^{3.0}$ $TCID_{50}$ per fish, and the NOR-92 isolate was delivered at doses of $10^{4.6}$, $10^{3.6}$, or $10^{2.6}$ $TCID_{50}$ per fish.

Maintenance and sampling of fish. Fish were maintained in a series of 27 l tanks in the BL-3 containment laboratory. The tanks were supplied with approximately 2 l min^{-1} specific-pathogen-free freshwater at an average temperature of 11°C. Effluent from the containment laboratory was treated with 5 mg l^{-1} chlorine for 30 min, a concentration previously determined to be in excess of that required to kill *Piscirickettsia salmonis* (authors' unpubl. data).

Mortalities were collected daily for 41 d post-injection. Survival curves were estimated using the Kaplan-Meier method, and a logrank test was used to compare the survival curves (Statview, Abacus Concepts, Inc., Berkeley, CA). Mean day to death was calculated for the fish that died during the 41 d period. At 41 d post-injection, the group that was injected with $10^{4.6}$ $TCID_{50}$ NOR-92 was still undergoing active mortality, and was maintained and observed for an additional 46 d. The

mean day to death for this group was also calculated for the 87 d period.

Post-mortem examinations were performed on all dead fish. Confirmation of infection with *Piscirickettsia salmonis* included reisolation of the organism from kidney tissue from 50% of the mortalties in each group and microscopic evaluation of stained tissue smears of 100% of the mortalities. *P. salmonis* was reisolated by aseptically removing a portion of the anterior kidney which was homogenized and diluted approximately 1:100 in MEM-10. The homogenate was inoculated into 4 replicate wells of a 96-well plate containing monolayers of CHSE-214 cells in MEM-10 supplemented with 100 U ml^{-1} penicillin and into 4 replicate wells without penicillin. Cells were incubated at 15°C and examined at 14 and 28 d for the appearance of CPE due to growth of *P. salmonis*. Smears of tissue from the kidney, liver, and spleen were made on 3 replicate slides. The slides were air dried, briefly heated, then fixed in absolute methanol for 5 min and stored at −20°C. Tissue smears from each fish were visually evaluated for the presence of *P. salmonis* by staining using the Giemsa method and confirmed by the indirect fluorescent antibody test (IFAT) described by Lannan et al. (1991).

Ten percent of the mortalities were screened for the presence of *Renibacterium salmoninarum*. Slides were randomly selected and a fluorescent antibody test performed as described by Pascho et al. (1991) using an anti-*R. salmoninarum* polyclonal antibody (Kirkgaard and Perry Laboratories, Inc., Gaithersburg, MD). The kidney samples were visually examined for the presence of the organism using fluorescence microscopy (50 fields of view at 1000×).

RESULTS

Each of the 3 isolates caused mortalities with signs of piscirickettsiosis. Moribund fish were dark, lethargic, and swam near the surface. Generally, the kidneys were swollen and the spleens enlarged, with no discrete lesions visible. Many fish had pale gills indicative of anemia. Some of the fish had ascites. Especially notable was the occurrence of extreme petechial hemorrhaging in the visceral fat and the pyloric caeca. There were no obvious differences in the gross pathology caused by the 3 isolates.

Piscirickettsia salmonis was recovered in cell culture from mortalities in each of the exposed groups. In cases where bacterial or fungal contamination of the CHSE-214 cell cultures prevented isolation of the organism, *P. salmonis* was identified by IFAT.

The first mortalities began on Day 13 post-injection (PI) in the group injected with LF-89 at a dose of $10^{5.0}$ $TCID_{50}$ $fish^{-1}$ (Fig. 1). The mean day to death in this group was 18 d PI, with peak mortality at 15 d PI, and the cumulative mortality was 97% at 41 d. In the groups exposed to 10-fold lower doses of LF-89, the initial mortalities, mean days to death, and peak mortalities were delayed (Table 1). The cumulative mortalities for the groups receiving doses of $10^{4.0}$ and $10^{3.0}$ $TCID_{50}$ $fish^{-1}$ were 91 and 57%, respectively. When survival times among groups of fish receiving the high and middle doses were compared using a logrank test, there was a significantly shorter survival time for fish receiving the high dose, and, similarly, the fish receiving the middle dose had shorter survival times than the fish injected with the low dose ($p < 0.0001$ for both comparisons).

Onset of mortality in the ATL-4-91 groups was delayed compared to the groups receiving the LF-89 isolate (Fig. 2). In fish injected with $10^{5.0}$ $TCID_{50}$ of the ATL-4-91 isolate, mortality began on Day 15 PI and peaked at 30 d PI, with a mean day to death of 27 d. Cumulative mortality was 92% in this group. A clear dose response pattern, similar to that seen in the groups of fish injected with the LF-89 isolate, occurred in the groups of fish injected with the ATL-4-91 isolate (Table 1). Cumulative mortalities reached 76 and 32% in the groups receiving $10^{4.0}$ and $10^{3.0}$ $TCID_{50}$ $fish^{-1}$, respectively. The median survival times were 27 and 35 d PI for the high and middle doses, and this differ-

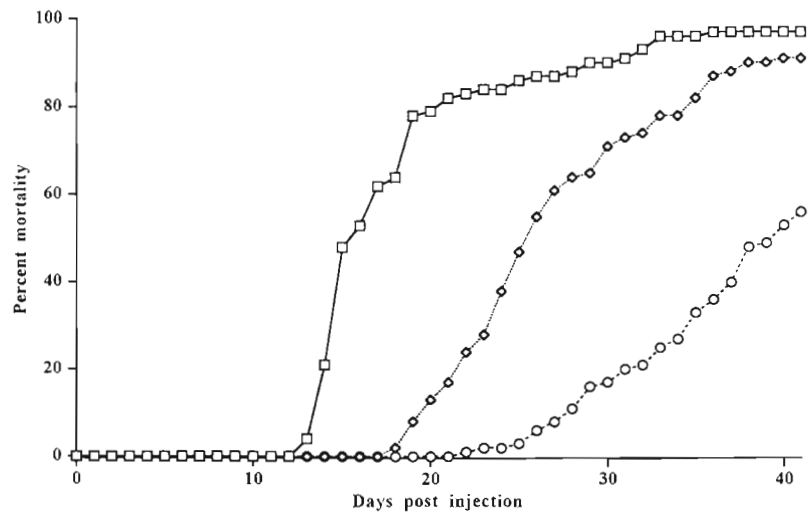

Fig. 1. *Piscirickettsia salmonis* infecting *Oncorhynchus kisutch*. Mean cumulative mortality of coho salmon injected intraperitoneally with different doses of the the LF-89 isolate from Chile. Doses used were (—□—) $10^{5.0}$ $TCID_{50}$, (----o----) $10^{4.0}$ $TCID_{50}$, and (····o····) $10^{3.0}$ $TCID_{50}$

Table 1. *Piscirickettsia salmonis* infecting *Oncorhynchus kisutch*. Mortality among groups of coho salmon held for 41 d following intrperitoneal injection of isolates

Isolate, source	Inoculum ($TCID_{50}$)	Initial mortality (d)	Peak mortality (d)	Mean day to death	Median survival time (d)	Cumulative mortality (%)
LF-89, Chile	$10^{5.0}$	13	15	18	16	97
	$10^{4.0}$	18	24	27	26	91
	$10^{3.0}$	22	38	33	40	57
ATL-4-91, British Columbia, Canada	$10^{5.0}$	15	30	27	27	92
	$10^{4.0}$	21	37	32	35	76
	$10^{3.0}$	26	38	36	–[b]	32
NOR-92, Norway	$10^{4.6}$	16	38	33	–[b]	41
	$10^{3.6}$	26	–[a]	33	–[b]	6
	$10^{2.6}$	8	–[a]	33	–[b]	2
Control	0	33	–[a]	34	–[b]	2

[a]Not calculated. [b]Unable to calculate from data; less than 50% died in this group

ence was significant ($p < 0.0001$). No statistical comparison was made with the low dose group because less than 50% of the fish had died at 41 d PI.

The groups inoculated with the NOR-92 isolate had lower cumulative mortalities and a slower onset and loss rate compared with the groups inoculated with the other isolates (Fig. 3). Fish receiving $10^{4.6}$ $TCID_{50}$ of this isolate began dying on Day 16 PI, and the daily number of mortalities increased slowly, with a peak at 38 d PI. At 41 d PI, the cumulative mortality reached 41%, with a mean day to death of 33 d PI. The observation of this group continued until 87 d PI, when the cumulative mortality had reached 70%. The mean day to death at this time was 41 d PI. In the group injected with $10^{3.6}$ $TCID_{50}$ $fish^{-1}$ the cumulative mortality was 6%, and only 2 fish injected with $10^{2.6}$ $TCID_{50}$ NOR-92 died over the 41 d period, for a cumulative mortality of 2% (Table 1). Death of the first fish in the latter group occurred on 8 d PI and was attributed to bacterial kidney disease (BKD). This fish had a large off-white lesion on the kidney, and examination of the kidney imprint stained with Geimsa, *Renibacterium salmoninarum* FAT and the *Piscirickettsia salmonis* IFAT revealed the presence of *R. salmoninarum*, but not *P. salmonis*. The second fish that died in this group at 33 d PI had piscirickettsiosis. The median survival time was not estimated for any of the groups injected with the NOR-92 isolate, as none of the groups had reached 50% mortality by 41 d PI.

Two of the 90 control fish died during the experiment (Fig. 3).

When survival data for similar doses of the different isolates were compared, it was found that fish injected with the LF-89 isolate had statistically shorter survival times than fish injected with the ATL-4-91 or NOR-92 isolates ($p < 0.0001$ for all comparisons). Additionally, fish injected with ATL-4-91 had significantly shorter survival times than fish exposed to the NOR-92 isolate ($p < 0.0001$ for all comparisons).

High mortalities in all treatment groups inoculated with isolate LF-89 prevented calculation of LD_{50} for that isolate. The LD_{50} values of $10^{3.4}$ $TCID_{50}$ $fish^{-1}$ for ATL-4-91 and $10^{4.6}$ $TCID_{50}$

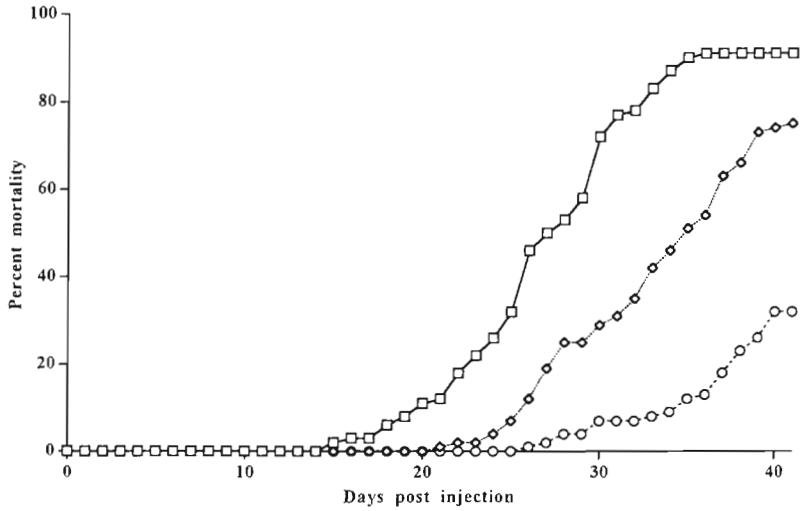

Fig. 2. *Piscirickettsia salmonis* infecting *Oncorhynchus kisutch*. Mean cumulative mortality of coho salmon injected intraperitoneally with different doses of the the ATL-4-91 isolate from British Columbia, Canada. Doses used were (—□—) $10^{5.0}$ $TCID_{50}$, (---◇---) $10^{4.0}$ $TCID_{50}$, and (····○····) $10^{3.0}$ $TCID_{50}$

fish^{-1} for NOR-92 were calculated based on the mortalities that occurred over the course of the 41 d study.

There were 6 fish out of the 900 (0.7%) in this study in which a lesion due to *Renibacterium salmoninarum* was noted on the kidney, with 1 case occurring in each of the following groups: LF-89 $10^{4.0}$ and $10^{3.0}$ $TCID_{50}$, ATL-4-91 $10^{4.0}$ $TCID_{50}$, NOR-92 $10^{3.6}$ and $10^{2.6}$ $TCID_{50}$, and the MEM-10 control group. In these few clinical cases, high numbers of *R. salmoninarum* were present and *Piscirickettsia salmonis* was not detected in kidney tissue impressions from these fish. Analysis of kidney impressions from the mortalities in the study indicated that, in addition to dying of *P. salmonis*, 6% of the fish harbored moderate or low infections with *R. salmoninarum* (40 or fewer bacterial cells in 50 fields observed at 1000×). No one treatment group was more severely affected than another, and because the *P. salmonis* IFAT results were used to confirm the specific cause of death, no fish were removed from the statistical analysis.

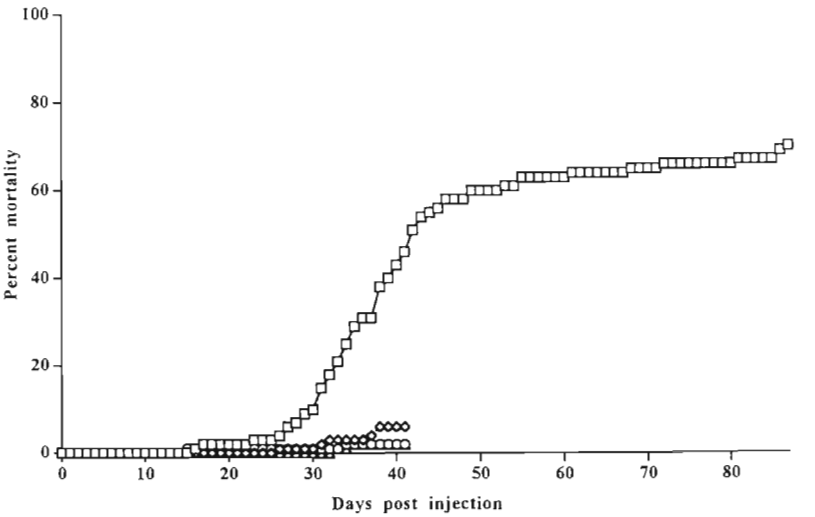

Fig. 3. *Piscirickettsia salmonis* infecting *Oncorhynchus kisutch*. Mean cumulative mortality of coho salmon injected intraperitoneally with different doses of the the NOR-92 isolate from Norway and of the negative control group. Doses used were (—□—) $10^{4.6}$ $TCID_{50}$, (----◇----) $10^{3.6}$ $TCID_{50}$, and (····○····) $10^{2.6}$ $TCID_{50}$, and (----△—) Control

DISCUSSION

Results from this study demonstrated statistically significant differences in the relative virulence of *Piscirickettsia salmonis* isolates from Chile (LF-89), British Columbia, (ATL-4-91) and Norway (NOR-92) for coho salmon. Isolate LF-89 caused the most rapid onset of mortality and significantly shorter survival times compared to isolates ATL-4-91 and NOR-92. There was a direct and significant relationship between dose and mortality, with delayed onset and decreased losses in the groups inoculated with lower concentrations of LF-89. The results in this study confirmed and extended challenge studies previously done in Chile with the LF-89 isolate (Garcés et al. 1991). In that study, doses of $10^{5.3}$, $10^{4.3}$ and $10^{3.3}$ $TCID_{50}$ were administered to 10 g coho salmon. Mortality in the high dose group began at 16 d post-injection, losses reached 100% by 33 d, and the mean day to death was 18 d. In both studies, the initial mortalities occurred later and the overall losses were less among groups that received the lower concentrations of LF-89. Results from our study and those from Garcés et al. (1991) did not allow the calculation of the LD_{50} for the LF-89 strain, but Smith et al. (1996) reported that the LD_{50} for the LF-89 isolate was $10^{2.8}$ $TCID_{50}$ ml^{-1} ($10^{1.5}$ $TCID_{50}$ fish^{-1}) in 16–17 g coho salmon. In the present study, 57% mortality had occurred by termination of the 41 d experiment in the group that received a dose of $10^{3.0}$ $TCID_{50}$ fish^{-1}, confirming that the LD_{50} values for the LF-89 strain are slightly below $10^{3.0}$ $TCID_{50}$ fish^{-1}.

Time to initial mortality among all groups inoculated with the isolate from British Columbia, ATL-4-91, was delayed compared to groups receiving equivalent doses of LF-89, and the survival time was significantly longer. The highest dose ($10^{5.0}$ $TCID_{50}$ fish^{-1}) of ATL-4-91 did cause severe losses (92% by 41 d), with a mean day to death of 27 d. As in the LF-89 groups, mortality was directly related to the concentration of bacteria administered. The LD_{50} of the ATL-4-91 isolate was calculated to be $10^{3.4}$ $TCID_{50}$ fish^{-1}. Brocklebank et al. (1993) reported that infection by a rickettsia-like agent (subsequently identified as the ATL-4-91 isolate of *Piscirickettsia salmonis*; G. Traxler, Pacific Biological Station, Nanaimo, British Columbia, pers. comm. 1998) caused a cumulative mortality of approximately 8% among Atlantic salmon and negligible losses among chinook salmon in affected netpens in British Columbia. Homogenates of kidney, liver and spleen from moribund fish injected intraperitoneally into healthy chinook salmon smolts caused mortality starting between 20 and 32 d post-injection, with 50 to 88% of the fish dead by 50 d post-injection (Brocklebank et al. 1993). The number of infectious units injected into the fish was not determined in that study; however, our laboratory studies confirmed that ATL-4-91 was virulent when delivered at a high concentration, although less so than the Chilean isolate, LF-89.

The Norway isolate, NOR-92, was the least virulent of the 3 isolates tested in this study, with only the high dose causing mortality that was significantly greater than that observed in the negative control aquaria. It was not possible to hold all of the groups beyond termination of the experiment; however, we were able to accommodate the high dose group until 87 d PI. Fish in this group had a relatively long onset to death, and at 41 d PI, the cumulative mortality was increasing. By the end of the extended period, cumulative mortality had reached 70%, with a mean day to death of 41 d PI. The lower mortality caused by this isolate in our laboratory study was consistent with the descriptions of piscirickettsiosis epizootics in netpen culture in Norway (Olsen et al. 1997). Although NOR-92 was isolated during a disease outbreak, the bacterium was believed to be directly responsible for only limited mortality, with higher losses incurred when other pathogens, such as infectious pancreatic necrosis virus, were involved (Olsen et al. 1997).

The reasons for the differences in virulence among the isolates are not clear, but our study, using controlled conditions and the same stock of fish, indicates that variation between the isolates is significant. While gross pathology was similar for all of the isolates, histological examination may provide further information. Olsen et al. (1997) reported that the pathology seen in Atlantic salmon infected with *Piscirickettsia salmonis* in Norway was generally comparable to that of infections occurring in salmon from Chile, British Columbia, and Ireland. However, certain signs, such as multifocal gill hyperplasia, dilation of the stomach, and inflammation and necrosis of the gut, that were reported by authors working with affected Chilean coho salmon (Branson & Nieto-Diáz-Muñoz 1991) were not seen in cases from Norway (Olsen et al. 1997) or Ireland (Rodger & Drinan 1993). Additionally, unlike that described in reports from Chile and British Columbia, extensive thrombosis was only observed occasionally among fish dying from piscirickettsiosis in Norway. Explanations for differences in the observed pathology include host factors, such as species and condition, as well as differences in the *P. salmonis* isolates themselves. An investigation of the comparative histopathology over the course of the disease may provide some insight into the mechanisms of virulence.

Previously, alignment of 16S rDNA sequences from the isolates of *Piscirickettsia salmonis* used in this study revealed a very high level (99.4%) of genetic similarity (Mauel 1996, Fryer & Mauel 1997). One isolate from Chile, EM-90, was distinguishable from these isolates in a PCR assay (Mauel et al. 1996), indicating that among certain isolates there are small, but detectable, genetic differences. Using convalescent salmonid antiserum, Kuzyk et al. (1996) observed no differences between the immunoreactive bands of the LF-89 strain and an isolate from British Columbia (BC-95). Although the isolates tested in this study are very closely related, our study was able to demonstrate significant biological differences among them.

Variation in virulence among isolates of rickettsia is not unprecedented. The existence of strains of *Rickettsia rickettsii* with varying virulence has long been recognized (Parker 1935, Price 1953), and McDade (1990) cites examples of strains of *Rickettsia prowazekii*, *Rickettsia akari* and *R. rickettsii*, the etiological agents of louse-borne typhus, rickettsialpox and Rocky Mountain spotted fever respectively, having differing virulence in experimental animals. Toxins, factors that effect adherence to the host cells, and mechanisms that enhance the ability of a pathogen to invade or survive within the host are known to affect virulence (Finlay & Falkow 1997). However, comparative studies of *R. rickettsii* strains (Anacker et al. 1984, 1986) have not identified specific differences in virulence factors (McDade 1990).

As researchers and aquaculturists become increasingly aware of rickettsia-like organisms in fish, the geographic and species distribution of these bacteria will become more defined. Consideration not only of the presence or absence of *Piscirickettsia salmonis*, but also of characteristics of isolates in an area or stock of fish, may be of value in making management decisions involving both farmed and wild fish. Development of diagnostic techniques capable of distinguishing unique antigens and/or genomic characteristics of the isolates will be important tools that will allow managers to make informed decisions concerning disease outbreaks and the movement of fish.

Acknowledgements. The authors are grateful to the Oregon Department of Fish and Wildlife for providing the coho salmon used in this study. We thank Dr G. Traxler of the Department of Fisheries and Oceans, Nanaimo, Canada, and Dr H. P. Melby of the National Veterinary Institute, Oslo, Norway, for providing isolates of *Piscirickettsia salmonis*. Finally, we greatly appreciate the advice and assistance of Cathy Lannan, Prudy Caswell-Reno and Margo Whipple. This work was supported by grant no. 58-319R-3-031 Amend. 4 from USDA OICD to Oregon State University, and by a grant from the Agricultural Research Foundation, Oregon State University. This is Oregon Agriculture Experiment Station Technical Paper no. 11340.

LITERATURE CITED

Alday-Sanz V, Rodger H, Turnbull T, Adams A, Richards RH (1994) An immunohistochemical diagnostic test for rickettsial disease. J Fish Dis 17:189–191

Anacker RL, Philip RN, Williams JC, List RH, Mann RE (1984) Biochemical and immunochemical analysis of *Rickettsia rickettsii* strains of various degrees of virulence. Infect Immun 44(3):559–564

Anacker RL, List RH, Mann RE, Wiedbrauk DL (1986) Antigenic heterogeneity in high- and low-virulence strains of *Rickettsia rickettsii* revealed by monoclonal antibodies. Infect Immun 51(2):653–660

Branson EJ, Nieto-Diáz-Muñoz D (1991) Description of a new disease condition occurring in farmed coho salmon *Onchorynchus kisutch* (Walbaum), in South America. J Fish Dis 14:147–156

Bravo S, Campos M (1989) Coho salmon syndrome in Chile. AFS/FHS Newsletter 17(3):3

Brocklebank JR, Evelyn TPT, Speare DJ, Armstrong RD (1993) Rickettsial septicemia in farmed Atlantic and chinook salmon in British Columbia: clinical presentation and experimental transmission. Can Vet J 34:745–748

Cusack R, Groman D, Jones S (1997) The first reported rickettsial infections of Atlantic salmon in eastern North America. In: VIII[th] International Conference 'Diseases of Fish and Shellfish', Abstract Book. Eur Assoc of Fish Pathol, Edinburgh, p 0–109

Cvitanich JD, Gárate N, Smith CE (1991) The isolation of a rickettsia-like organism causing disease and mortality in Chilean salmonids and its confirmation by Koch's postulate. J Fish Dis 14:121–145

Finlay BB, Falkow S (1997) Common themes in microbial pathogenicity revisited. Micro Mol Biol Rev 61(2):136–169

Fryer JL, Mauel MJ (1997) The rickettsia: an emerging group of pathogens in fish. Emerg Infect Dis 3(2):137–144

Fryer JL, Lannan CN, Garcés LH, Larenas JJ, Smith PA (1990) Isolation of a rickettsia-like organism from diseased coho salmon *Onchorynchus kisutch* in Chile. Fish Pathol 25(2): 107–114

Fryer JL, Lannan CN, Giovannoni SJ, Wood ND (1992) *Piscirickettsia salmonis* gen. nov. sp. nov., the causative agent of an epizootic disease in salmonid fishes. Int J Syst Bacteriol 42(1):120–126

Garcés LH, Larenas JJ, Smith PA, Sandino S, Lannan CN, Fryer JL (1991) Infectivity of a rickettsia isolated from coho salmon (*Onchorynchus kisutch*). Dis Aquat Org 11:93–97

Jones SRM, Markham RJF, Groman DB, Cusack RR (1998) Virulence and antigenic characteristics of a cultured Rickettsiales-like organism isolated from farmed Atlantic salmon *Salmo salar* in eastern Canada. Dis Aquat Org 33: 25–31

Kuzyk MA, Thornton JC, Kay WW (1996) Antigenic characterization of the salmonid pathogen *Piscirickettsia salmonis*. Infect Immun 64(12):5205–5210

Lannan CN, Winton JR, Fryer JL (1984) Fish cell lines: establishment and characterization of nine cell lines from salmonids. In Vitro 20:671–676

Lannan CN, Ewing SA, Fryer JL (1991) A fluorescent antibody test for detection of the rickettsia causing disease in Chilean salmon. J Aquat Anim Health 3:229–234

Mauel MJ (1996) Evidence for molecular diversity of *Piscirickettsia salmonis*. PhD thesis, Oregon State University, Corvallis

Mauel MJ, Giovannoni SJ, Fryer JL (1996) Development of polymerase chain reaction assays for detection, identification and differentiation of *Piscirickettsia salmonis*. Dis Aquat Org 26:189–195

McDade JE (1990) Evidence supporting the hypothesis that rickettsial virulence factors determine the severity of spotted fever and typhus group infections. Ann NY Acad Sci 590:20–26

Olsen AB, Melby HP, Speilberg L, Evensen Ø, Hastein T (1997) *Piscirickettsia salmonis* infection in Atlantic salmon *Salmo salar* in Norway—epidemiological, pathological and microbiological findings. Dis Aquat Org 31:35–48

Reed LJ, Muench H (1938) A simple method of estimating fifty percent endpoints. Am J Hyg 27:493–497

Rodger HD, Drinan EM (1993) Observation of a rickettsia-like organism in Atlantic salmon *Salmo salar* L., in Ireland. J Fish Dis 16:361–369

Palmer R, Ruttledge M, Callanan K, Drinan E (1997) A piscirickettsiosis-like disease in farmed Atlantic salmon in Ireland—isolation of the agent. Bull Eur Assoc Fish Pathol 17(2):68–72

Parker RR (1935) Rocky Mountain spotted fever: results of ten years' prophylactic vaccination. J Infect Dis 57:78–98

Pascho RJ, Elliott DG, Steufert JM (1991) Brood stock segregation of spring chinook salmon *Onchorynchus tshawytscha* by use of the enzyme-linked antibody technique (ELISA) and the fluorescent antibody technique (FAT) affects the prevalence and levels of *Renibacterium salmoninarum* infection in progeny. Dis Aquat Org 12: 25–40

Price WH (1953) The epidemiology of Rocky Mountain spotted fever. I. The characterization of strain virulence of *Rickettsia rickettsii*. Am J Hyg 58:248–268

Smith PA, Contreras JR, Garcés LH, Larenas JJ, Oyanedel S, Caswell-Reno P, Fryer JL (1996) Experimental challenge of coho salmon and rainbow trout with *Piscirickettsia salmonis*. J Aquat Anim Health 8:130–134

Editorial responsibility: David Bruno, Aberdeen, Scotland, UK

Submitted: May 28, 1998; Accepted: October 26, 1998
Proofs received from author(s): January 22, 1999