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Comparison of the enzyme-linked immunosorbent assay (ELISA) and the fluorescent antibody test (FAT) for measuring the prevalences and levels of *Renibacterium salmoninarum* in wild and hatchery stocks of salmonid fishes in Alaska, USA

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ABSTRACT: The enzyme-linked immunosorbent assay (ELISA) and the fluorescent antibody test (FAT) were compared for their sensitivity in detection of *Renibacterium salmoninarum* (Rs) in kidney tissues of Alaskan salmonids. The ELISA appeared to be more sensitive in detecting Rs infections. The FAT did not detect Rs in 80 % of the ELISA-positive samples but was positive for Rs in 28 % of the samples that were ELISA-negative. This contradiction may have been due to low-level washover of Rs cells from smears containing large numbers of Rs cells when slides containing multiple samples were rinsed in a common vessel during the FAT procedures. The FAT routinely did not detect infections in Rs-positive fish the tissues of which produced a mean ELISA optical density value ≤0.173, and inconsistently detected infections in fish with ELISA values >0.173 but <0.978. The 0.978 optical density was the mean ELISA value at which the FAT routinely detected Rs-positive fish. Based on the ELISA results, Rs occurred in only 9 % of the Alaskan Pacific salmon tested in both wild (85 %) and hatchery (81 %) stocks. The very high stock prevalences and levels of Rs antigen detected in wild trout *Oncorhynchus* spp., char *Salvelinus* spp., and grayling *Thymallus arcticus* having no clinical signs of bacterial kidney disease suggest these species may be somewhat resistant hosts and important freshwater reservoirs of Rs.

INTRODUCTION

Bacterial kidney disease (BKD) caused by *Renibacterium salmoninarum* (Rs) continues to be a serious disease concern in salmonid culture worldwide (Fryer & Sanders 1981, Sanders & Barros 1986, Elliott et al. 1989). Control of both the disease and the dissemination of the agent remains difficult due to the ability of the bacterium to be vertically transmitted from parents to progeny inside the eggs (Bullock et al. 1978, Evelyn et al. 1984, 1986b, Lee & Evelyn 1989, Pascho et al. 1991) and to survive intracellularly within host cells (Young & Chapman 1978, Gutenberger et al. 1991), thus reducing bacterial exposure to the host immune defenses and any therapeutants. An effective vaccine is not yet available (Elliott et al. 1989) so avoidance of horizontally transmitted Rs (Mitchum & Sherman 1981, Bell et al. 1984) and of vertically transmitted Rs is still a

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primary management strategy. In addition to a water supply free of Rs, avoidance has largely been attempted by using eggs from certified Rs-free fish stocks or by manipulation of Rs-positive broodstocks to reduce vertical transmission to the progeny. One such manipulation has been injection of antibiotics into maturing female broodfish producing an accumulation of therapeutic drug levels in the yolk of the embryo (Groman & Klontz 1983, Bullock & Leek 1986, Evelyn et al. 1986a, Armstrong et al. 1989a, Brown et al. 1990). Another alternative has been the elimination or segregation of eggs and fry spawned from Rs-positive parents (Pascho et al. 1991). The identification of Rs-positive parents requires rapid and sensitive detection methods that can be applied on a large scale. Several methods have been compared for the detection of the causative agent in carrier broodstocks (Pascho et al. 1987, Elliott et al. 1989, Sakai et al. 1989). Due to the ease of application, the most common method has been examination of kidney smears by the fluorescent antibody test (FAT) which replaced the less sensitive Gram stain (Bullock & Stuckey 1975, Bullock et al. 1980). More recently, the enzyme-linked immunosorbent assay (ELISA) has been shown to be the most sensitive, accurate, quantitative, and easily automated test for large-scale detection of Rs soluble antigens in salmonids (Pascho & Mulcahy 1987, Pascho et al. 1987, Turaga et al. 1987, Sakai et al. 1989, Hsu et al. 1991, Pascho et al. 1991).

The purpose of this study was to evaluate the FAT and an ELISA, both based on commercially available reagents, for screening broodstocks for the presence (and control) of Rs (Pascho et al. 1991) in Alaskan salmonids. Eggs from Rs-positive broodstocks were to be destroyed. In addition, we wished to develop a data base of samples tested by both methods that was larger than any currently reported in the literature and which would facilitate the interpretation of Rs data in other laboratories where this particular ELISA is used. Finally, we wished to determine the prevalence of Rs antigen in Alaskan salmonids and to compare the prevalences in hatchery and wild salmonid stocks.

MATERIALS AND METHODS

Test fish and tissue samples. Kidney tissues tested by both the ELISA and the FAT were from mature salmon of 5 Pacific species (see Table 1) collected from southeast Alaska during 1988 and 1989. Tissues from trout (*Oncorhynchus mykiss, O. clarki*), char (*Salvelinus namaycush, S. fontinalis, S. malma*), and Arctic grayling (*Thymallus arcticus*) collected from several watersheds in southeast Alaska during 1988 to 1991 were examined primarily by the ELISA (see Table 4). Except for 2 samples noted in Table 4, all of these fish were adults. Nearly all fish examined were either freshwater residents or anadromous species that had returned to fresh water to spawn. The exceptions were 276 chinook salmon *O. tshawytscha* and 54 steelhead *O. mykiss* that matured in saltwater netpens prior to collection of kidney samples.

Wild stocks were defined as self-perpetuating fish populations that, except in a few cases, were of complete natural origin. However, for some of these wild stocks minor enhancement had occurred previously; eggs from these wild stocks had been taken, and reared fry had been transplanted back into the natal systems. In many of these cases, eggs and progeny had been maintained in isolation and supplied with depurated or fish-free water while at a hatchery. The number of trout, char, and grayling stocks examined was low, with only 4 hatchery stocks of rainbow trout being tested. The rest of the stocks were wild, with no known history of enhancement except for the brook trout and possibly one other stock of rainbow trout that had been transplanted from hatcheries in the Pacific Northwest and intermountain areas of the U.S. during the early 1950s.

Samples of anterior and posterior kidney tissues each weighing at least 0.5 g were excised by use of sterile wooden disposable or iodophor-disinfected (3 %) utensils from individual adult mature salmon. Tissues from individual fish were placed into 60 ml (2 oz) sterile plastic bags and either frozen at -5 to -10 °C for later shipment or sent directly to the fish pathology laboratories for processing within the Commercial Fisheries Management and Development (CFMD) Division of the Alaska Department of Fish and Game (ADF&G). Occasionally for some of the smaller adult trout, char, and grayling species, either 2 to 5 whole kidneys or liver and kidney from individual fish were pooled to provide 0.3 to 3.0 g of sample material. Undiluted samples were homogenized for about 20 s using a Stomacher (Tekmar, Cincinnati, OH, USA).

FAT. The direct and indirect FAT procedures used were similar to those described by the American Fisheries Society Fish Health Section (Amos 1985). Smears of individual undiluted homogenized tissue samples were made with a drop of phosphate buffered saline (PBS, pH 7.2) in individual wells on glass slides coated with a teflon 10-well template. After air drying, slides were heat-fixed followed by fixation in anhydrous methanol for 5 min. All rinses were in distilled water. Incubation and rinse times were 5 min each, and stained smears were drained before mounting with coverslip in P-phenylenediamine immunofluorescence mounting fluid (pH 8.6). Prior to use, all reagents and distilled water were filtered through sterile

0.45 µm porosity cellulose nitrate or polysulfone filters and all fixations and rinses were done in coplin jars, each holding 6 slides. The direct test utilized commercially prepared goat anti-Rs fluorescein isothiocyanate (FITC) conjugate (Kirkegaard and Perry Laboratories, Inc., Gaithersburg, MD, USA) at a working dilution of 1:120 (v/v) in PBS containing a 1:150 (v/v) dilution of rhodamine counterstain (Difco Laboratories, Detroit, MI, USA). For the indirect test, we used a chicken anti-Rs primary antiserum (Battelle Labs, Sequim, WA, USA) diluted to 1:200 (v/v) in PBS, followed by commercially prepared goat anti-chicken FITC conjugate (Cappel, Organon Teknika Corp., Durham, NC, USA) diluted 1:200 and containing rhodamine diluted as above. Kidney smears from adult chinook and coho *Oncorhynchus kisutch* salmon, known to be Rs-infected by previous broodstock examinations, were used as positive controls. Negative FAT controls consisted of the same Rs-positive smears incubated with PBS and normal serum. Slides containing the negative and positive controls were each rinsed in separate coplin jars to avoid any potential cross-contamination.

A minimum of 30 fields was examined on each sample well at 1000× magnification with a Nikon microscope equipped for immunofluorescence work. Results were rated from 0 to 5 according to the following criteria: 0 = negative with no Rs cells seen in 30 fields; 1= 1 to 5 typical Rs cells observed in 30 fields with examination of up to 100 fields in an attempt to find a second organism; 2 = 6 to 50 Rs cells observed in 30 fields; 3 = 51 to 150 Rs cells observed in 30 fields; 4 = more than 150 Rs cells in 30 fields with less than 200 observed in any given field; 5 = more than 200 Rs cells observed in any given field.

ELISA. After FAT smears were made, the undiluted kidney homogenates were processed for the ELISA as described by Meyers et al. (1993) using commercially available reagents (Kirkegaard and Perry Laboratories, Inc., Gaithersburg, MD, USA). Samples having an optical density value ≥0.095 were considered positive for the Rs antigen.

Statistical analysis. Statistical analyses of ELISA data are described in Meyers et al. (1993) including the correction statistic for interassay comparison of data by Pascho et al. (1987). This statistic was used to correct for any minor fluctuations in optical density values of test fish samples due to new reagents or day-to-day changes in equipment performance. This statistic was not applied to the trout, char, and grayling optical densities from samples collected in 1990 and 1991.

Statistical comparisons of Rs antigen prevalences in Tables 3 & 5 were made using arcsine transformation of proportions and compared to probability values in a 2-tailed distribution table (Sokal & Rohlf 1969).

RESULTS

Comparison of ELISA and FAT

The comparative data in Tables 1, 2 & 3 and those from nonanadromous salmonids collected in 1988 and 1989 in Table 4 were produced from 37 ELISAs, each done on a different day. Optical density values from 14 (38 %) of these assays required correction using the statistic by Pascho et al. (1987). The mean (0.0815) of the negative control samples (n = 397) remained the same whether the correction statistic was applied or not.

Results from chinook, coho, pink *Oncorhynchus gorbuscha*, chum *O. keta*, and sockeye *O. nerka* salmon kidneys examined by both the ELISA and the FAT during 1988 and 1989 are presented in Table 1. The detection prevalences for both tests were compared. A total of 10451 kidneys were tested by the ELISA of which 991 (9 %) were positive for Rs antigen (Table 1). Among these kidneys, 5818 were tested by both the ELISA and the FAT; 651 (11 %) were positive by the ELISA and only 183 (3 %) were positive by the FAT. Among the ELISA-positive kidneys only 132 (20 %) were positive by the FAT, but among the FAT-positive kidneys 51 (28 %) were ELISA-negative. The FAT positives that proved ELISA-negative always had low-level FAT ratings of 1 or 2. Exceptions in which the FAT and ELISA results correlated more closely were observed in samples of sockeye salmon examined in 1988 (Table 1).

Table 2 provides the mean FAT ratings and mean ELISA optical density values of samples that were Rs-positive in both assays. Kidney samples with a mean ELISA optical density value of about 1.0 or greater were consistently detected by the FAT at a mean rating of about 2. Although the FAT occasionally detected organisms in samples with lower ELISA optical density values, it did not do so routinely. The mean ELISA optical density values for Rs-positive samples that were not detected by the FAT (Table 2) indicated that the FAT was routinely missing Rs-positive fish with a mean ELISA value of 0.173 or less and was inconsistently detecting fish with ELISA values >0.173 but <0.978. Generally, FAT detection occurred with high ELISA values (≥1.0) but the FAT rating was neither proportional nor consistent. Occasionally the FAT would not detect Rs cells in kidneys having very high ELISA values (≥2.0).

Rs prevalences in Alaskan fish stocks by the ELISA

The prevalences of Rs antigen in female and male chinook, coho, and pink salmon from 1988 and 1989 were compared to determine if significant differences

Table 1. Comparison of ELISA and FAT results for the detection of *Renibacterium salmoninarum* (Rs) in 5 species of Alaskan Pacific salmon (KS = chinook; SS = coho; PS = pink; CS = chum; RS = sockeye) collected during 1988 and 1989. Mean optical density values ≥ 0.095 were considered positive for the Rs antigen. nd: not done

Species	Year	No. of stocks	% Rs detection by: ELISA	FAT	FAT pos., ELISA neg.[a]	ELISA pos., FAT neg.[b]	ELISA and FAT pos.[c]	
KS	1988	7	8 (238/2955)	2 (65/2955)	23/65	196/238	42/238	(18 %)
	1989	13	11 (247/2276)					
			43 (112/258)[d]	9 (24/258)	1/24	89/112	23/112	(21 %)
SS	1988	9	5 (93/1719)	1 (23/1719)	8/23	78/93	15/93	(16 %)
	1989	10	11 (222/2074)					
			31 (56/182)[d]	11 (20/182)	3/20	39/56	17/56	(30 %)
PS	1988	2	35 (34/98)	0 (0/98)	0	34/34	0/34	(0)
	1989	5	10 (26/257)					
			6 (3/51)[d]	0 (0/51)	0	3/3	0/3	(0)
CS	1988	1	11 (7/66)	0 (0/66)	0	7/7	0/7	(0)
	1989	6	19 (59/314)					
			43 (52/122)[d]	0 (0/122)	0	52/52	0/52	(0)
RS	1988	6	15 (56/367)	14 (51/367)	16/51	21/56	35/56	(63 %)[e]
	1989	4	3 (9/325)	nd				
Totals			9 (991/10 451)					
			11 (651/5818)[d]	3 (183/5818)	51/183 (28 %)	519/651 (80 %)	132/651	(20 %)

[a] Prevalence of FAT-positive samples that were negative by ELISA
[b] Prevalence of ELISA-positive samples that were negative by the FAT
[c] Prevalence of ELISA-positive samples also positive by the FAT
[d] Subsampled groups of fish tested by both the FAT and ELISA
[e] Many kidneys with high numbers of Rs cells allowing better detection by the FAT and more potential cross-contamination of Rs-negative smears

occurred (Table 3). Chinook salmon females had a higher prevalence of Rs antigen than males ($p < 0.001$). Coho salmon males had a higher prevalence of Rs antigen than females ($p < 0.05$) but no significant differences in antigen prevalences occurred between the sexes for pink salmon. When the data from all 3 species were pooled, females had a greater Rs antigen prevalence than males ($p < 0.05$).

Table 2. Mean ELISA and FAT values for detection of *Renibacterium salmoninarum* (Rs) in 5 species of Alaskan Pacific salmon (KS = chinook; SS = coho; PS = pink; CS = chum; RS = sockeye) during 1988 and 1989. Mean ELISA optical density values ≥ 0.095 were considered positive for the Rs antigen

Species	Year	Mean FAT/ mean ELISA[a]	Ranges (FAT/ELISA)	Mean ELISA for neg. FAT[b]	Range
KS	1988	1.7/0.817	1-5/0.095-2.8	0.204	0.095-2.5
	1989	1.7/0.803	1-4/0.106-2.6	0.148	0.095-1.4
SS	1988	2.0/0.929	1-4/0.110-2.1	0.187	0.095-1.2
	1989	2.6/1.69	1-5/0.469-4.0	0.141	0.095-0.83
PS	1988	-	-	0.147	0.095-0.53
	1989	-	-	0.496	0.15 -0.80
CS	1988	-	-	0.107	0.095-0.12
	1989	-	-	0.105	0.095-0.18
RS	1988	2.4/0.962	1-5/0.096-4.0	0.198	0.095-1.26
Totals		2.1/0.978	1-5/0.095-4.0	0.173	0.095-2.5

[a] Mean FAT rating and ELISA optical density values in those kidney samples positive for Rs by both the ELISA and FAT in Table 1
[b] Mean ELISA optical density values for Rs-positive kidney samples that were not FAT-positive in Table 1

Table 3. ELISA-based prevalence of *Renibacterium salmoninarum* (Rs) soluble antigen in male and female chinook (KS), coho (SS) and pink (PS) salmon in Alaska during 1988 and 1989. Mean optical density values ≥0.095 were considered positive for the Rs antigen

Species	Year	Rs prevalence (%) Male	Female
KS[a]	1988	4.4 (45/1024)	9.9 (187/1892)
	1989	7.9 (68/859)	11.6 (138/1193)
Subtotal		6.0 (113/1883)	10.5 (325/3085)
SS[b]	1988	4.7 (15/321)	5.6 (78/1398)
	1989	13.8 (51/370)	8.5 (134/1574)
Subtotal		9.6 (66/691)	7.1 (212/2972)
PS	1989	11.1 (6/54)	20.5 (16/78)
Total[c]		7.0 (185/2628)	9.0 (553/6135)

[a] Statistically significant difference ($p<0.001$) between male an female Rs antigen prevalences
[b] Statistically significant difference ($p<0.05$) between male and female Rs antigen prevalences
[c] Statistically significant difference ($p<0.05$) between Rs antigen prevalences of males and females in the pooled data of all 3 species

The prevalences of Rs antigen in wild and hatchery stocks of Pacific salmon and various other Alaskan salmonid species examined from 1988 to 1991 are presented in Tables 4 & 5. Among the Pacific salmon, there was no significant difference ($p<0.05$) in the percentages of Rs-positive stocks whether of hatchery (38/47, 81 %) or of wild (23/27, 85 %) origin.

Among the wild stocks of trout, char, and grayling, all had detectable Rs antigen, often with very high prevalences within any single population (Table 5). The 4 hatchery stocks, though positive for Rs, had lower prevalences within each stock. The correction statistic was not used for adjusting the ELISA optical density values for the nonanadromous salmonid stocks collected in 1990 and 1991 because it would not have significantly changed the prevalence data presented in Table 4 or have altered the number of Rs-positive stocks reported in Table 5. This is because the optical densities were generally very high positive values, and not near the negative-positive threshold. Hence, there were few 'borderline' fish to be affected by minor adjustments due to the correction statistic.

DISCUSSION

Some improvement in agreement between FAT and ELISA results may have been achieved by examination of more fields. However, other investigators have shown a similar lack of agreement of FAT results among laboratories (Armstrong et al. 1989b), and FAT results also disagreed with ELISA results (Pascho et al. 1987, Hsu et al. 1991, Pascho et al. 1991) and with results of other detection methods more sensitive than the FAT, but less so than the ELISA (Lee & Gordon 1987, Griffiths et al. 1991), when 50 to 100 fields were examined. Consequently, examination of more than 30 fields by the FAT did not appear worthwhile in this study. Very good agreement (99 %) between FAT and a monoclonal ELISA was reported by Rockey et al. (1991). However, their Rs cell rating criteria indicated high enough numbers of Rs cells in the kidneys examined to allow detection by FAT regardless of the number of fields examined. High numbers of Rs cells in a number of our 1988 sockeye salmon kidneys in Table 1 also accounted for better FAT agreement with ELISA.

Positive FAT samples that were ELISA-negative could have been due to cross-contamination of negative kidney samples by Rs cells dislodged from kidney smears containing high numbers of Rs cells. Cross-contamination is conceivable because of the low-level FAT ratings observed for all of these kidneys and because these kidneys tended to occur on slides on which there were smears containing high numbers of Rs cells (Table 1). An alternative explanation is that the ELISA-negative kidneys were actually infected with Rs cells below the detectable limit of the ELISA and that by chance a few Rs cells were observed by the FAT. We did not do any additional testing of these kidneys since there has been no other method available as sensitive as the ELISA for Rs detection. Testing for the possibility of washover was also not done, but washover could be eliminated by using individual rinsing protocols and by using 1 smear per slide. However, use of such methods would not have been practical for routine FAT examination of the large sample numbers in this study.

The failure of the FAT to detect Rs cells in occasional kidney samples having very high ELISA optical density values (≥2.0) may have been due to 2 limitations of the FAT. First, a positive FAT reaction requires intact organisms rather than soluble antigen, and, second, the distribution of Rs cells within an infected kidney may not always be homogeneous. Uneven (focal) distribution of organisms in the kidney and the very small tissue sample homogenized for the FAT could have resulted in insufficieat numbers of Rs cells being present in the smears to ensure their detection (10^6 Rs cells ml^{-1} of tissue homogenate are needed for detection by FAT; Bullock et al. 1980). Uneven distribution of Rs cells may also explain the inconsistent and poorly correlated FAT ratings when compared to ELISA optical density values, reported above. Uneven distribution of Rs cells would likely become a greater source of error in larger salmonid species due to their larger kidneys

Table 4. ELISA-based prevalence of *Renibacterium salmoninarum* (Rs) soluble antigen in several trout, char, and grayling stocks in Alaska during 1988 to 1991 All stocks were wild except where noted. Mean optical density values ≥ 0.095 were considered positive for the Rs antigen. BY: brood year

Fish stock	% Prevalence			
	1988	1989	1990	1991
Brook trout *Salvelinus fontinalis*				
Crystal Lake	100 (12/12)	96.0 (48/50)	–	–
Green Lake	–	–	–	100 (54/54)
Salmon Lake	–	–	–	93.2 (55/59)
Upper Dewey Lake	–	95.1 (39/41)	–	–
Cutthrout trout *Oncorhynchus clarki*				
Auke Lake	–	–	–	11.1 (2/18)
Connell Lake	–	31.6 (6/19)	–	–
Margaret Lake	100 (2/2)	–	–	–
Dolly Varden *Salvelinus malma*				
Sheep Creek	–	–	–	100 (9/9)
Grayling *Thymallus arcticus*				
Chena River	–	–	–	88.5 (54/61)
Herman Lake	–	–	–	98.1 (51/52)
Lake trout *Salvelinus namaycush*				
Paxson Lake	–	93.3 (28/30)	15.2 (12/79)	–
BY 1989 progeny	–	–	0 (0/60)	
Rainbow trout *Oncorhynchus mykiss*				
Big Lake[a]	51.1 (23/45)	–	0 (0/60)	–
Blue Lake	–	–	62.1 (36/58)	–
Carlanna Lake	–	–	80.0 (4/5)	–
Harriet Hunt Lake	–	–	100 (3/3)	–
Round Lake	86.5 (45/52)	–	–	–
Sashin Creek	95.7 (66/69)	–	–	–
Sashin Lake	70.1 (40/57)	100 (13/13)	–	–
Swanson R[a]	–	–	10.0 (6.60)	–
BY 1989 progeny	–	–	0 (0/59)	–
Upper Silvis Lake	–	–	100 (9/9)	–
Steelhead trout *Oncorhynchus mykiss*				
Admiralty Creek	–	100 (1/1)	50.0 (1/2)	–
Falls Creek	–	–	–	10.0 (1/10)
Ketchikan Creek[b]	–	–	0 (0/24)	9.1 (1/11)
Sashin Creek[c]	–	–	12.5 (1/8)	5.6 (1/18)
Sashin Creek[a, c]	–	–	0 (0/8)	8.7 (4/46)

[a] Hatchery stock
[b] Feral hatchery stock
[c] Saltwater netpen reared to sexual maturity

or in fish recovering from infections where Rs cells may no longer be present in high numbers. In recovering fish, immune complexes containing soluble Rs antigen are, however, still present in the kidney (Sami et al. 1992), thus making them ELISA-positive. Also, the major focus of Rs cells may be in a tissue other than the kidney (Hendricks & Leek 1975, Hoffmann et al. 1984, Ferguson 1989) but large quantities of soluble antigen, secreted by the Rs cells (Kaattari et al. 1989), are likely to accumulate homogeneously in the kidney melanomacrophage centers, as occurs with other antigens (Ellis et al. 1976, Nelson et al. 1985). Thus, the kidney would be strongly ELISA-positive but FAT-negative.

Our laboratory results suggested that the ELISA method for the detection of Rs antigen in kidney tissues was superior to the FAT in its sensitivity and in its suitability for use with large sample numbers. The discrepancy between our ELISA and FAT results indicated that the majority of low- and mid-level Rs-positive fish would have been missed by FAT and that a significant number of Rs-negative fish may possibly be identified as falsely positive using the FAT. Other disadvantages of using the FAT as a screening

Table 5. Numbers of wild and hatchery salmonid stocks in Alaska examined by ELISA that were positive for the soluble antigen of *Renibacterium salmoninarum* (Rs) from 1988 to 1991: 74 stocks of 5 Pacific salmon and 24 stocks of trout, char, and grayling. Mean optical density values ≥0.095 were considered positive for the Rs antigen

Species	Rs-positive stocks[a]	Range in prevalence
Chinook salmon		
Wild	3/4 (75 %)	0–90 %[b]
Hatchery	11/11 (100 %)	0–59 %
Coho salmon		
Wild	5/7 (71 %)	3–26 %
Hatchery	10/11 (91 %)	0–22 %
Sockeye salmon		
Wild	9/10 (90 %)	0–45 %
Hatchery	2/2 (100 %)	15–16 %
Chum salmon		
Wild	1/1 (100 %)	5 %
Hatchery	5/10 (50 %)	0–51 %
Pink salmon		
Wild	5/5 (100 %)	7–50 %
Hatchery	10/13 (77 %)	0–40 %)
Rainbow and steelhead trout		
Wild	9/9 (100 %)	10–100 %
Hatchery	4/4 (100 %)	0–51 %
Cutthroat trout (wild)	3/3 (100 %)	11–100 %
Brook trout (wild)	4/4 (100 %)	93–100 %
Lake trout (wild)	1/1 (100 %)	0–93 %
Dolly Varden (wild)	1/1 (100 %)	100 %
Grayling (wild)	2/2 (100 %)	89–98 %

[a] No significant difference ($p < 0.05$) between wild and hatchery stocks of Pacific salmon
[b] Multiple samples are represented in which an Rs-positive stock may have 0 % prevalence of Rs detected in 1 or more years

method include greater error in interpretation, particularly during long periods of sample viewing or when results from more than 1 viewer are combined. Contrary to the above, FAT combined with the filtration method is both sensitive and quantitative when applied to ovarian fluid samples and it uses single sample processing without the potential for cross-contamination (Elliott & Barila 1987).

Although the prevalences of Rs antigen were essentially equal in both male and female fish for pink salmon, this was not so for chinook and coho salmon. As mentioned before, coho and chinook salmon yielded results that were opposite to each other regarding Rs prevalence between the sexes. Pooling of the data, including that for pink salmon, showed, however, that overall, females had a significantly higher occurrence of the antigen. Whether females are more susceptible to Rs infection or are more immunologically compromised needs to be determined. A difference in Rs susceptibility between male and female brook trout was reported by Bullock et al. (1971), but specific details were not given.

There is considerable controversy as to whether hatchery practices serve to perpetuate and disseminate certain fish diseases in wild stocks. In Alaskan Pacific salmon the prevalence of Rs-infected wild and hatchery stocks was essentially the same. It should be noted that initial selection of fish stocks for ELISA testing was done at random and not because they were suspected of having BKD. All Alaskan hatchery stocks of Pacific salmon and most nonanadromous salmonids presently in use originated from indigenous wild stocks. Consequently, many of the diseases present in hatchery fish were already present in the donor wild stocks or in the resident wild fish occurring in the water supplies to some of the hatcheries. Several wild fish stocks that were Rs-positive had never experienced any known human intervention until tissue samples were collected for this study. Also interesting is the higher percentage of Rs-positive wild trout, char, and grayling stocks. In many of these stocks, previous FAT examinations had failed to detect any Rs oganisms. In those few stocks where Rs was detected by the FAT, gross inspection of infected individuals revealed no external or internal clinical signs of BKD. Also, only 1 or 2 fish within a sample were low-level FAT-positive. In contrast, 100 % were positive by the ELISA, often with very high optical density values (unpublished CFMD Pathology Laboratory reports). Consequently, the FAT and ELISA results did not correlate in the nonanadromous salmonids (unlike the findings for anadromous salmon in Table 2). Kidney lesions and subclinical infections that could be detected by both the FAT and ELISA were commonly observed in returning adult coho and chinook salmon. This suggests that Rs infections were more active in the Pacific salmon than in the trout, char, or grayling. Previous studies have also demonstrated that wild stocks of trout (Mitchum et al. 1979), Atlantic salmon *Salmo salar* (Paterson et al. 1979), Arctic char *Salvelinus alpinus*, and lake trout *Salvelinus namaycush* (Souter et al. 1987) can have clinical as well as subclinical Rs infections. Indeed, in contrast to our findings, there is one example in which considerable mortality in Rs-infected wild brook trout *Salvelinus fontinalis* occurred (Mitchum et al. 1979). On the otherhand Canadian scientists have, like us, also observed that wild trout and char typically carry the Rs organism without showing clinical signs. Their view is that trout and char are better adapted to the organism than are Pacific salmon and consequently are more resistant to BKD (Dr Trevor Evelyn, Pacific Biological Station, Nanaimo, B.C.,

Canada pers. comm.). This view is supported by other reports where steelhead and rainbow trout were most resistant to Rs infection, followed by brown trout *Salmo trutta* and brook trout, whereas chinook, coho, and sockeye salmon were generally more susceptible than trout (Bullock et al. 1971, Sanders et al. 1978, Mitchum et al. 1979, Bullock & Wolf 1986). Japanese studies have also shown that rainbow trout and native Japanese char *Salvelinus pluvius* are more resistant to Rs infection than coho salmon and other salmon species (Sakai et al. 1991).

In Alaska, wild trout, char, and grayling may be important reservoirs of Rs for both wild and hatchery stocks of anadromous Pacific salmon. Nonanadromous salmonids are the major native resident fishes in many watersheds and hatchery water supplies statewide. Therefore, it is not surprising that the Rs agent appears to be ubiquitous in feral salmonid stocks, sometimes occurring at very high levels in the most pristine of wild fish stocks. It may be argued that hatchery stocks are not the major source of diseases for wild stocks but rather they are more likely at risk of disease from exposure to potential disease agents carried by wild stocks. This is particularly likely in the hatchery environment where high densities of fish increase the risk of epizootics following introduction of a fish pathogen. This is also a concern in the natural environment; at least one documented study showed that BKD was transmitted from wild fish to transplanted hatchery trout (Mitchum & Sherman 1981). It also is obvious that intensive fish culture certainly can amplify the pathogen carrier rate within a given stock of hatchery fish. However, if practices within the hatchery and the transplanting of Rs-positive hatchery fish are controlled, wild and other hatchery stocks of fish should not be adversely affected. Additionally, there is further need to control horizontal transmission of Rs to hatchery fish where feasible by depurating hatchery water supplies containing resident salmonid fishes.

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