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Source: Journal of Wildlife Diseases, 30(4) : 577-580

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-30.4.577

Journal of Wildlife Diseases, 30(4), 1994, pp. 577–580

Relationship Between Resistance of Salmonids to Furunculosis and Recovery of *Aeromonas salmonicida* From External Mucus

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ABSTRACT: Fish were sampled at the Ed Weed State Fish Hatchery (South Hero, Vermont, USA) in September 1992. *Aeromonas salmonicida* was common, with concentrations as high as 10^5 to 10^7 colony-forming units per gram of mucus, and readily recovered from most mucus samples obtained from furunculosis-sensitive populations of brook trout (*Salvelinus fontinalis*), lake trout (*Salvelinus namaycush*), and Atlantic salmon (*Salmo salar*). The pathogen was the predominant microorganism and accounted for greater than 85% of the total number of bacteria isolated from the mucus of these fish. By comparison, *A. salmonicida* was recovered only from two rainbow trout (*Oncorhynchus mykiss*), and bacterial frequencies did not exceed 10^3 colony-forming units per gram of mucus. The pathogen was not recovered from the mucus of steelhead (*O. mykiss*) or Rome brown trout (*Salmo trutta*) selectively bred for resistance to furunculosis, even though there was widespread contagion throughout the hatchery and fish were cultured on a common, unprotected water supply.

Key words: Furunculosis, *Aeromonas salmonicida*, resistance, mucus, bacterial prevalence, *Oncorhynchus mykiss*, *Salmo trutta*, *Salmo salar*, *Salvelinus fontinalis*, *Salvelinus namaycush*.

The most successful development of furunculosis-resistant strains of trout was conducted by the New York State Department of Environmental Conservation at its Fish Disease Unit in Rome, New York (USA). Wolf (1953) challenged 11 strains of brook trout (*Salvelinus fontinalis*) and seven strains of brown trout (*Salmo trutta*) from New England (USA) and eastern Canada, with *Aeromonas salmonicida* and found substantial variation in mortality. Ehlinger (1964) selectively bred survivors between Wolf's strains that had demonstrated intermediate or low mortality and produced resistant strains of both brook trout and brown trout, and improved the culture of salmonids throughout New York (Ehlinger, 1977).

One strain each of furunculosis-resistant brook trout and brown trout still are maintained at Rome (John Schachte, pers. comm.). Resistance of Rome trout to furunculosis is widely accepted by many practicing fish culturists, and these fish are used in many programs throughout the New England and Mid-Atlantic regions of the USA. Unfortunately, detailed information does not exist on actual hatchery performance during natural exposure to the pathogen. Our objective was to compare the recovery of *A. salmonicida* from mucus of furunculosis-resistant brown trout to recovery from more sensitive salmonids.

From the second week of July until the end of September 1992, a furunculosis epizootic among production lots of salmon and trout occurred at the Ed Weed State Fish Hatchery, South Hero Vermont (USA) (44°40′N, 37°20′E). All fish were cultured on a common water supply derived from Lake Champlain, Vermont. Because of mechanical problems associated with the ultraviolet irradiation unit at this facility, non-irradiated water was used to culture fish. Shortly thereafter, furunculosis mortality was diagnosed by the state fish health pathologist among populations of brook trout, landlocked Atlantic salmon (*Salmo salar*), and lake trout (*Salvelinus namaycush*). Mortality was not observed among more resistant populations of rainbow trout (*Oncorhynchus mykiss*), steelhead (*Oncorhynchus mykiss*), and resistant brown trout from the Rome hatchery.

Presence of *A. salmonicida* was determined by culture from the mucus of 27 randomly sampled fish per production lot. Sample size was selected to ensure 95% confidence for detecting a 10% prevalence of infection (Amos, 1985). Samples were

collected and evaluated as described by Cipriano et al. (1992). Briefly, mucus was diluted 1:10 (weight/volume) in sterile phosphate-buffered saline (pH = 7.2). Serial $\log_{10}$ dilutions of each sample were prepared in phosphate buffer and 0.01 ml aliquots of each dilution were dropped onto Coomassie Brilliant Blue agar (Cipriano and Bertolini, 1988). Plates were incubated for 72 hr at 18 to 20 C. When possible, bacterial numbers were estimated in those dilutions counts containing 10 to 30 colonies; however, it sometimes was necessary to identify bacteria from dilutions containing less than 10 colonies. Individual colonies, within the dilutions that were counted, were subcultured onto Bacto-Tryptic Soy Agar (Difco Laboratories, Detroit, Michigan, USA) and identified by the methods of MacFaddin (1980).

Aeromonas salmonicida was readily cultured from mucus of juvenile brook trout, Atlantic salmon, and lake trout that were sustaining furunculosis mortality. Mortality was most severe in brook trout; the pathogen was recovered from 23 fish (Table 1) and composed 94% of all bacteria identified from this group (Table 2). The pathogen also was detected in the mucus from 20 Atlantic salmon and 13 lake trout and accounted for 98% and 85% of the total number of bacterial identifications, respectively. Among these fish, *A. salmonicida* was the predominant microorganism and had extensively colonized the mucus. The upper limits of pathogen frequencies in lake trout, Atlantic salmon, and brook trout attained values as high as 8.8×10^5, 1.10×10^7 and 4.7×10^7 colony-forming units (cfu) per gram of mucus, respectively (Table 1).

By comparison, *A. salmonicida* was detected only in mucus samples from two rainbow trout and composed just 14% of the total number of bacteria identified from these fish. The numbers of *A. salmonicida* on external surfaces of rainbow trout were low, compared with that for the three species of fish already mentioned, and did not exceed 3.8×10^3 cfu/g of mucus. The pathogen was not recovered from the mucus of any steelhead or brown trout sampled. Bacteria identified from these fish included *Staphylococcus* spp., *Acinetobacter* spp., *Moraxella* spp., *Comamonas terrigenia*, several pseudomonads, and, to a lesser extent, *Aeromonas hydrophila* (Table 2).

These bacteria were consistent with normal bacterial flora commonly associated with salmonid hosts (Horsley, 1973).

The non-lethal procedures used in this study allowed direct recovery of *A. salmonicida* from mucus of infected fish. We assumed that subsequent estimation of bacterial numbers was related to differential sensitivities of specific salmonid hosts to furunculosis (Cipriano and Heartwell, 1986). Elucidation of external bacterial prevalences, therefore, produced a relative measure of susceptibility to infection. The inability to recover *A. salmonicida*, especially from external surfaces of brown trout selectively bred for resistance to furunculosis, is evidence for an unknown mechanism for resistance in the mucus of fish.

The natural barrier activity of skin and mucous proteases (Hjelmeland et al., 1983) are critical in the initial defense against infection. Cipriano and Heartwell (1986) also reported that mucus from certain species of salmonids could precipitate extracellular antigens produced by *A. salmonicida*. The extent of precipitation was species-dependent and appeared related to level of resistance to disease. Brown trout from the Rome hatchery selectively bred for resistance to furunculosis, had high levels of this mucus precipitin activity. Factors in external mucous secretions of resistant fish, therefore, already have been implicated to moderate the host response to furunculosis (Cipriano and Heartwell, 1986).

Our results were consistent with the existence of defense mechanisms in salmonid mucus that can inhibit infection caused by *A. salmonicida*. *Aeromonas salmonicida* caused extensive mortality and was easily

TABLE 1. Recovery of *Aeromonas salmonicida* from the mucus of subsamples of six different production lots of juvenile salmonids maintained on a common water supply at the Ed Weed State Fish Hatchery, South Hero, Vermont, September 1992.

Production lot	Number of fish infected[a]	Colony forming units per gram	
		Mean	Maximum
Brook trout	23	2.4×10^6	4.7×10^7
Atlantic salmon	20	8.6×10^5	1.1×10^7
Lake trout	13	7.7×10^4	8.8×10^5
Rainbow trout	2	1.7×10^2	3.8×10^3
Steelhead	0	ND[b]	ND
Brown trout	0	ND	ND

[a] Mucus from 27 randomly sampled fish per species.
[b] Not determined.

recovered from brook trout, Atlantic salmon, and lake trout. However, the pathogen was difficult to recover from rainbow trout and was not isolated from steelhead; these latter trout are considered to be naturally resistant to *A. salmonicida* (Blake and Clark, 1931). Furthermore, the bacterium was not recovered from the mucus of any brown trout from the Rome hatchery, which were selectively bred for resistance to furunculosis (Ehlinger, 1964).

Although discrete populations of fish do not cohabit the same production raceways at the Ed Weed State Fish Hatchery, resistant brown trout were adjacent to other sensitive hosts. In addition, all fish were cultured in a common water supply derived from Lake Champlain, and the ultraviolet irradiation system, originally designed to ensure pathogen-free water, was inoperative for several weeks before the initial onset of disease. Opportunity existed for exposure to *A. salmonicida* either through a contaminated water supply or by horizontal transmission from other lots of fish. Although it is possible that the brown trout never were exposed to *A. salmonicida*, the high degree of contagion throughout the remainder of the facility makes this unlikely. More likely, the inability to recover *A. salmonicida* from brown trout or most of the other resistant hosts is evidence that these fish actively produce mucous substances that deter external colonization by the pathogen and, thereby, preclude infection.

Because the expression of disease involves many interactive processes between host, pathogen, and environment, Wolf (1953) warned that selective breeding could enhance resistance but not confer absolute immunity. Our investigations support his statement, and the present data should not be misconstrued to indicate that the brown trout studied herein, or other

TABLE 2. Percent distribution of bacteria identified from mucus of subsamples of six different juvenile salmonid production lots at the Ed Weed State Fish Hatchery, South Hero, Vermont, September 1992.

Bacterial species	Salmonid production lot[a]					
	Brook trout	Atlantic trout	Lake trout	Rainbow trout	Steelhead trout	Brown trout
Aeromonas salmonicida	402 (94)[b]	253 (98)	150 (86)	4 (14)	0 (0)	0 (0)
Aeromonas hydrophila	0 (0)	0 (0)	0 (0)	0 (0)	1 (6.3)	1 (3.7)
Acinetobacter spp.	21 (4.9)	0 (0)	10 (5.7)	12 (41)	2 (12)	5 (18)
Comamonas terrigenia	0 (0)	0 (0)	6 (3.4)	3 (10)	0 (0)	2 (7.4)
Moraxella spp.	1 (0.2)	0 (0)	2 (1.1)	0 (0)	2 (12)	5 (18)
Pseudomonas diminuta	0 (0)	0 (0)	0 (0)	0 (0)	2 (12)	2 (7.4)
Pseudomonas fluorescens	0 (0)	0 (0)	1 (0.6)	0 (0)	0 (0)	0 (0)
Pseudomonas pseudoalcaligenes	0 (0)	0 (0)	0 (0)	0 (0)	1 (6.2)	0 (0)
Staphylococcus spp.	3 (0.7)	4 (1.6)	6 (3.4)	10 (34)	8 (50)	12 (44)
Total number of bacteria identified	427	257	175	29	16	27

[a] Twenty-seven fish were sampled per production lot.
[b] Number of isolates from this bacterial species (percent of all bacteria isolated) for this production lot.

resistant salmonids, cannot become infected with *A. salmonicida*. All lots of fish held at the Ed Weed State Fish Hatchery were sampled at quarterly intervals throughout the production year. Although *A. salmonicida* was prevalent in the sensitive species of salmonids described herein (data not presented), the pathogen was detected from the mucus of only five of 27 brown trout sampled during the final examination in March 1993.

We are grateful to the fish culture staff at the Ed Weed State Fish Hatchery for their support, cooperation, and assistance throughout this study.

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Received for publication 8 September 1993.