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Innate susceptibility differences in chinook salmon *Oncorhynchus tshawytscha* to *Loma salmonae* (Microsporidia)

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ABSTRACT: *Loma salmonae* (Putz, Hoffman and Dunbar, 1965) Morrison & Sprague, 1981 (Microsporidia) is an important gill pathogen of Pacific salmon *Oncorhynchus* spp. in the Pacific Northwest. Three strains of chinook salmon *O. tshawytscha* were infected in 2 trials with *L. salmonae* by feeding of macerated infected gill tissue or per os as a gill tissue slurry. Intensity of infection was significantly higher in the Northern stream (NS) strain as compared to the Southern coastal (SC) and a hybrid (H) strain derived from these 2 strains. Both wet mount and histological enumeration of intensity of infection demonstrated strain differences. Survival in the NS strain was significantly lower than the other strains. The NS strain may represent a naive strain and be less able to mount an effective immune response against the parasite.

KEY WORDS: *Loma salmonae* · Microsporidia · Innate susceptibility

INTRODUCTION

Loma salmonae (Putz, Hoffman and Dunbar, 1965) Morrison & Sprague, 1981 infects endothelial cells of Pacific salmon *Oncorhynchus* spp. and is considered a serious gill pathogen in the Pacific Northwest (Kent & Poppe 1998, Shaw & Kent 1999). This microsporidian parasite provokes hypertrophy of host cells in which spores are being formed. These parasite-host cell complexes are termed xenomas and found predominately in the gills, but also throughout vascularized tissue of the host. Xenomas can occlude blood vessels, rupture and result in a severe inflammatory response by the host when xenomas rupture releasing spores (Hauck 1984, Kent et al. 1989, Speare et al. 1989). These released spores can infect the same host forming another xenoma or be released to the environment and ingested by a new host (Shaw et al. 1998). Once in the alimentary canal a *L. salmonae* spore is stimulated to extrude a polar filament which pierces a host cell, injecting the infective sporoplasm. Shaw et al. (1998) suggested this sporoplasm may enter the blood stream through the lamina propria, eventually reaching and infecting an endothelial cell to form the xenoma.

Genetic variation between strains of fish from different geographic origins has been demonstrated for protist infections. Clayton & Price (1992) noted both inter- and intraspecific variation in resistance to the ciliate *Ichthyophthirius multifiliis* Fouquet, 1876. Numerous studies have examined differential susceptibility of salmonid strains to the myxosporean *Ceratomyxa shasta* Noble, 1950 and haemoflagellate *Cryptobia salmositica* Katz, 1951 (Bower & Evelyn 1988, Ibarra et al. 1991, 1992, 1994, Bower 1995, Bower et al. 1995, Forward et al. 1995, Forward & Woo 1996). Both Chevassus & Dorson (1990) and Wiegertjes et al. (1996) provide extensive reviews of genetic variation of resistance to disease in fish. However, no information exists on variation of strains of fish to infection by microsporidia.

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Production of 3 strains of pen-reared (farmed) chinook salmon *Oncorhynchus tshawytscha* by the aquaculture industry in British Columbia, Canada, resulted in anecdotal reports of different susceptibility to *Loma salmonae* in netpens. We investigated this further by infecting these strains of chinook salmon with *L. salmonae* under controlled laboratory conditions.

MATERIALS AND METHODS

Fish husbandry. Three strains of chinook salmon were obtained from Sea Springs Hatchery, Duncan, British Columbia, Canada, which receives only well water and has no history of *Loma salmonae*. The first strain is termed Southern coastal (SC) after wild stocks of Big Qualicum River, Vancouver, Island, Canada. The second strain is termed Northern stream (NS) after wild stocks entering the Yukon river in the Yukon Territory, and the last was a hybrid (H) of these 2 strains. All fish were the same year class and were transferred to the Pacific Biological Station in Nanaimo, British Columbia, where they were held for 3 wk to adapt to laboratory conditions. Fish received dechlorinated fresh water (14 to 16°C) in flow-through tanks and were fed daily to satiation a diet of commercial fish-feed. During handling, fish were anesthetized with tricaine methanesulfonate (MS-222).

Preparation of parasite. The protocol of Kent et al. (1995) was followed for preparation of macerated gill tissue and the protocol of Shaw et al. (1998) was followed for preparation of an infective gill tissue slurry.

Exposure protocol. Susceptibility of the chinook strains was examined in 2 separate trials. In the first trial 70 fish per strain (average total length and weight SC: 11.0 cm, 11.6 g; NS: 9.3 cm, 6.9 g; H: 12.9 cm, 21.0 g) were fin-clipped, placed in separate 726 l deep-oval tanks (fresh water, 18°C), food withheld for 2 d, and then fed 23.8 g of macerated *Loma salmonae* infected gill tissue per tank over 3 d, using the same inoculum for each tank. On Day 4 all fish were placed in 1 tank and 17 negative controls per strain were added. Fish were examined for presence of *L. salmonae* only by histology 27 d later because of high mortality. Every 12 h during this trial, presence of *L. salmonae* was also recorded by wet mount examination of gills in fish that had died, as the gills of these fish were too autolysed for examination by histology. Viscera from 20 fish per strain were preserved for histological examination to evaluate systemic infections by *L. salmonae*.

For Trial 2, a group of 120 fish per strain (average total length and weight SC: 12.3 cm, 22.0 g; NS: 9.5 cm, 10.0 g; H: 11.6 cm, 18.0 g) were individually identified with pit tags (BioSonics Inc., Seattle, WA), and held in tanks for 4 wk to heal from tagging. A total of 100 fish per strain were exposed to *Loma salmonae* per os (infective gill tissue slurry introduced directly into the stomach) at 1.1×10^6 spores per fish, and placed in 1 deep-oval (726 l) tank. One day later, 10 negative controls per strain were added. Every 12 h prevalence and intensity of *L. salmonae* were recorded by wet mount in fish that died during the trial. Intensity was not recorded in gills of fish that were too autolysed for accurate evaluation. At 43 d (approximately 50% mortality overall), all remaining fish were examined by histology and wet mount.

Evaluation methods. At sampling fish were killed with an overdose of MS-222 and total length and weight of each fish was taken. The first left gill arch of each fish was examined by wet mount for *Loma salmonae* and intensity enumerated from the average of 3 random counts of the number of xenomas per ×100 field of view (1.5 mm diameter) and converted to xenomas mm^{-2} gill tissue. The remainder of the gills were placed in Davidson's solution (Humason 1979) along with complete viscera and a section of dorsal muscle for histology. All these tissues were processed using standard histological techniques. Histological sections of these tissues were stained with hematoxylin and eosin and examined at ×100. Intensity was enumerated in histological sections using the same methods described above for wet mounts.

Before fish were exposed, the left gill arch of 30 fish from each strain was examined by wet mount and histology for any infection by *Loma salmonae*.

Statistics. All prevalence data was tested using a chi-square test for goodness of fit. Intensity data was log transformed $x' = \log(x+1)$, then tested with an analysis of variance (ANOVA), followed by a Tukey Honestly Significantly Different (THSD) test. Histological and wet mount enumeration were compared as an estimate of parasite abundance in Trial 2. Time to death and survival was also tested for Trial 2 first by ANOVA, then by THSD test. Weights and lengths from all strains were tested with a Spearman rank correlation against their respective intensity of infection.

RESULTS

All fish examined before exposure were negative for *Loma salmonae* infection. We observed clinical signs of lethargy, flared opercula, darkening of the tail or body in moribund fish and gill pallor, ascites, hemorrhagic pyloric caeca, petechial hemorrhaging in the gills, skin and fins and numerous xenomas on the gills in dead fish from both trials. There were no significant differences between strains in prevalence of infection; however, intensity was significantly higher ($p < 0.001$;

Table 1. *Loma salmones* infecting *Oncorhynchus tshawytscha*. Prevalence and intensity of infection in 3 strains of chinook salmon after exposure by feeding infected gill tissue (Trial 1) or administration per os (Trial 2) of an infected gill slurry. Controls represent cohabitating but non-experimentally infected fish

Screening method	Prevalence[a]				Xenoma intensity[b]			
	Southern coastal	Northern stream	Hybrid	Control	Southern coastal	Northern stream	Hybrid	Control
Histology (Trial 1)[c]	54/55	43/43	56/62	0/51	1.7 (0.0–4.5)	1.9 (1.0–6.4)	0.9 (0.0–10.2)	0.0 (0.0–0.0)
Histology (Trial 2)[d]	59/59	35/36	51/51	0/30	1.0 (0.1–6.0)	4.4 (0.4–20.9)	1.0 (0.0–5.0)	0.0 (0.0–0.0)
Wet mount (Trial 2)	58/59	36/36	51/51	0/30	6.1 (0.0–17.0)	18.1 (6.4–38.5)	5.1 (1.2–13.9)	0.0 (0.0–0.0)

[a]No. positive/no. examined; [b]average xenomas mm^{-2} (range); [c]samples taken 27 d post-infection; [d]samples taken 43 d post-infection

1 outlier omitted in Trial 1) in the NS strain for both trials (Tables 1 & 2). Days to death did not differ significantly ($p = 0.152$) between strains of chinook. Histological or wet mount enumeration did not affect the ability to detect strain differences, although more xenomas were detectable by wet mount. Survival in the NS strain was also significantly lower ($p < 0.05$) (Fig. 1). *L. salmonae* was distributed in all organs sampled with some previously undocumented xenomas in the pyloric caeca, dermis, and mesentery (Table 3). There was no significant correlation ($p < 0.05$) with weight or length of fish and intensity of infection.

DISCUSSION

In general most fish are susceptible to initial infections but differ in their ability to limit or destroy pathogens (Wiegertjes et al. 1996). The terminology 'resistant' and 'susceptible' therefore, is always relative, and thus a reproducible method of exposure is vital in demonstrating differences between strains. Our first trial employed feeding of macerated gill tissue and larger fish may have consumed more gill tissue, leading to a higher intensity of infections. High mortalities in both trials were likely due to infection by *Loma salmonae* as fish exhibited typical clinical signs, e.g. gill pallor and hemorrhaging, ascites, and numerous xenomas in the gills (Hauck 1984, Kent & Poppe 1998). Trial 1 was terminated earlier than Trial 2 (i.e. 27 vs 43 d post-infection respectively) due to high mortalities. Mortalities in Trial 1 may have occurred earlier than Trial 2 as a result of the higher water temperature (18°C vs 14 to 16°C respectively). Development of *L. salmonae* proceeds more rapidly at higher water temperatures (Beaman et al. 1999), and more severe disease has been noted in coho salmon *Oncorhynchus kisutch* in the spring when water temperatures are rising (Kent et al. 1989).

Although both trials demonstrated differences between the chinook strains, per os exposure of fish (Trial 2) to equal doses of *Loma salmonae* is the consistent, reproducible method. For Trial 2 (per os expo-

Table 2. *Loma salmones* infecting *Oncorhynchus tshawytscha*. Average xenoma intensity in gills from mortalities of 3 strains of chinook salmon infected per os with *L. salmonae* during a 43 d holding period at 14 to 16°C (Trial 2). Mortalities with extremely autolysed gills (unreadable) were not evaluated

Strain (no. of mortalities evaluated)	Xenoma intensity[a]
Southern coastal (19)	6.6 (2.3–13.1)
Northern stream (19)	16.2 (2.4–42.1)
Hybrid (18)	7.3 (3.5–11.9)

[a]Avg. xenoma intensity mm^{-2} (range) in mortalities over 43 d

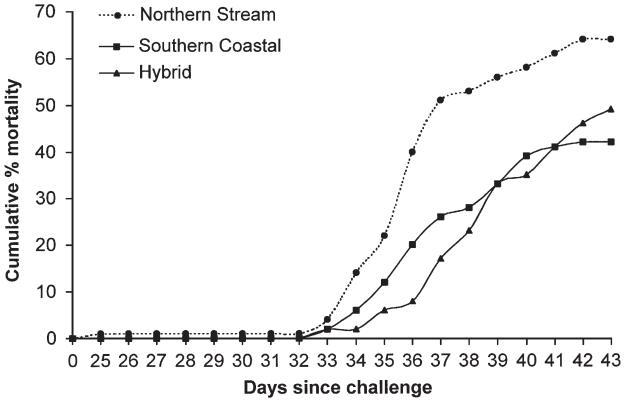

Fig. 1. *Loma salmones* infecting *Oncorhynchus tshawytscha*. Cumulative percent mortality in 3 strains of chinook salmon over time after per os infection with *L. salmonae* and being held for 43 d at 14 to 16°C

Table 3. *Loma salmones* infecting *Oncorhynchus tshawytscha*. Organ distribution of *L. salmonae* in 3 strains of chinook salmon after exposure by feeding infected gill tissue (Trial 1) or administration per os (Trial 2) of an infected gill slurry. Values are percentage of fish showing infection in organ indicated. –: not determined

Trial	Strain	No. fish examined	Tissue								
			Gill	Heart	Kidney	Spleen	Liver	Intestine	Pyloric caeca	Dermis	Mesentery
1[a]	Southern coastal	20	100.0	20.0	0.0	30.0	1.0	–	–	–	–
	Northern stream	20	100.0	10.0	0.0	40.0	0.0	–	–	–	–
	Hybrid	20	100.0	20.0	35.0	30.0	0.0	–	–	–	–
2[b]	Southern coastal	58	100.0	39.7	31.0	32.8	1.7	1.7	5.2	1.7	0.0
	Northern stream	51	100.0	68.6	96.1	49.0	1.7	5.9	9.8	7.8	9.8
	Hybrid	36	97.2	16.7	58.3	8.3	0.0	8.3	5.5	2.8	5.5

[a]Samples taken 27 d post-infection; [b]samples taken 43 d post-infection

sure) intensity of infection was significantly higher and survival significantly lower in the NS strain compared to the other 2 strains. Bower et al. (1995) found that coho salmon from the Kitimat River were highly susceptible to *Cryptobia salmositica* compared with coho from Big Qualicum River. They postulated that this may be because the parasite and it's vector are not enzootic to the Kitimat River. Shaw et al. (2000) found that wild NS chinook sampled for *L. salmonae* were negative while many other Pacific salmon spp. were positive. Although they sampled only 60 chinook from the Yukon river, their results and ours suggest this strain may represent a naive strain. The northern distribution of NS chinook may exceed the natural distribution of *L. salmonae*.

Natural disease resistance in fish is a complex interaction of genetic and non-genetic factors that may have multiple components (Chevassus & Dorson 1990). Price (1985) discusses genetic factors that include: barriers (i.e. skin and mucous); effects on pathogens of the host environment (i.e. gastric secretions, pH of blood, deficiencies in compounds required by the pathogen, natural antitoxins); and humoral and/or cellular immune responses. Non-genetic factors may include pathogen abundance, virulence, and temperature effects on pathogen development (Ibarra et al. 1994). Differences in disease resistance between strains of fish have been linked to variation in levels of mucus precipitin activity (Cipriano et al. 1994), plasma lipid components (Maita et al. 1998), antibody production (Strønsheim et al. 1994a,b), complement (Røed et al. 1990, 1992, 1993), and respiratory burst activity in head kidney macrophages (Balfry et al. 1994).

We found that significantly fewer NS chinook survived than SC or H and that there were no differences in time to death. Survivability is considered the best criterion for evaluating resistance in strains of fish as this represents the sum of all host-parasite interactions (Wassom & Kelly 1990). Ibarra et al. (1991) noted that only 13% of the *Ceratomyxa shasta* resistant strain of rainbow trout died compared with 90% of the susceptible strain, although they did not find any difference in time to death (Ibarra et al. 1994). Studies of *C. shasta* suggest 2 interacting mechanisms influencing parasite establishment: success of initial invasion by the parasite and the ability of the fish to mount an effective immune response (Ibarra et al. 1994). *Loma salmonae* may be unable to infect endothelial cells of resistant hosts and/or establish itself because of the immune response of the host. Macrophages are believed to be the primary defense mechanism of fish against microsporidia and phagocytosis and destruction of spores by macrophages has been described for several fish-infecting microsporidian species (Dyková & Lom 1980, Canning & Lom 1986). Shaw (1999) found that macrophages of Atlantic salmon *Salmo salar*, which are resistant to *L. salmonae*, phagocytosed more spores than susceptible chinook salmon. Although he did not find significant differences between SC and NS chinook strains using this assay, he postulated other immune mechanisms (e.g. respiratory burst) might account for this.

The NS fish in both experiments were smaller than the other strains, and thus the possibility that size affected the intensity of infection had to be considered. However, we found no relationship between size and intensity within the 3 strains.

In conclusion, *Loma salmonae* continues to be a serious gill pathogen in wild and farmed Pacific salmon. We found a chinook strain endemic to the Yukon river to be more susceptible to infection by the parasite when compared to a local strain from the Big Qualicum river, confirming the observations of fish farmers from netpen farms. The NS strain may represent a naive strain which is unable to mount as effective an immune response to the parasite. Future breeding experiments can further clarify these differences and investigate the underlying mechanisms.

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