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NOTE

Comparative susceptibility of two races of *Salmo salar* (Baltic Lule river and Atlantic Conon river strains) to infection with *Gyrodactylus salaris*

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ABSTRACT: The susceptibility of various races of salmonids towards infections with the skin parasitic monogenean *Gyrodactylus salaris* Malmberg, 1957, differs markedly. Norwegian and Scottish salmon strains are known as extremely susceptible to infection, whereas Baltic salmon races such as the Neva strain (Russian origin) and the Indals river (Swedish origin) salmon have been characterized as relatively resistant. However, the status of the many other Baltic strains has remained unknown. The present study reports on the susceptibility of the Baltic salmon from the Swedish river Lule. It was shown that this strain is susceptible to infection but to a lesser extent than the Scottish salmon. Further studies showed that injection of immuno-suppressants (dexamethasone) greatly increased population growth of *G. salaris* on Scottish salmon but not on the Baltic salmon. Mucous cell density on fins differed between strains, and a general trend to decreased cell density on infected fish 8 wk post-infection, compared to uninfected fish, was observed. The largest decrease in mucous cell density following infection was seen in the most resistant fish. After administration of immuno-suppressants, this decrease in mucous cell density was inhibited in the Scottish salmon but not in the Baltic salmon. Thus, there seems to be a relationship between the fishes' ability to discard mucous cells and the ability to resist infections with *Gyrodactylus salaris*. Although the Lule salmon seems more susceptible to infection compared to previous reports on the Neva salmon, the results support the notion that Baltic salmon strains are generally more resistant than East Atlantic salmon.

KEY WORDS: Atlantic salmon · *Salmo salar* · Conon river · Lule river · *Gyrodactylus salaris* · Susceptibility · Mucous cells · Dexamethasone

The skin parasitic monogenean *Gyrodactylus salaris* Malmberg, 1957, has devastated Norwegian salmon stocks due to the extreme susceptibility of this salmon race (Johnsen & Jensen 1991, Mo 1994) to infection. Studies have shown that Scottish strains of the Atlantic salmon are quite susceptible as well (Bakke & MacKenzie 1993). In contrast, salmon from the Russian Neva river draining into the Baltic are known to be relatively resistant (Bakke et al. 1990). Likewise, the Baltic salmon from the Indals river are also less susceptible (Bakke et al. 2002). This follows the hypothesis that the parasite has been present in the Baltic region since the last glacial period and has exerted heavy selection pressure on the local salmon race (Bakke et al. 2002). However, the Baltic salmon race comprises a vast number of different strains homing to different rivers (Nilsson et al. 2001), and the susceptibility status of these, except the Neva and Indals river strains, has not been reported. Therefore we have conducted a comparative study on the susceptibility to infection with *G. salaris* of Baltic salmon from the Lule river and Atlantic salmon from the Scottish river Conon.

Materials and methods. ***Fish:*** *Scottish salmon:* Eyed eggs of the Scottish strain from the Conon river were purchased from the Danish hatchery DVC-Randers in Jutland (western part of Denmark). Fish were subsequently transported to our laboratory at the Royal Veterinary and Agricultural University (KVL) and used for infection experiments 6 mo post-hatching. The Conon river salmon had a body length of between 7.8 and 8.9 cm and a body weight of between 8.1 and 8.4 g.

Baltic salmon: Eyed eggs of the Baltic strain were obtained from the Swedish hatchery (Vattenfall, Luleå, northern Sweden). Eggs of both strains were brought to the Bornholm salmon hatchery (Nexø, Bornholm), where hatching and rearing of larvae were conducted under pathogen-free conditions in recirculated freshwater. Fish were subsequently transported to our laboratory at the Royal Veterinary and Agricultural University (KVL) and used for infection experiments 6 mo post-hatching. The corresponding length of the Lule river salmon was between 9.5 and 10.1 cm with a body weight ranging between 10.2 and 10.7 g.

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Parasites: *Gyrodactylus salaris* on Norwegian salmon collected in the river Lærdalselva in Norway were brought to our laboratory at Frederiksberg, Denmark and used for the establishment of a laboratory population on Scottish Conon salmon kept in 200 l aquaria at 13°C.

Corticosteroid treatment of fish: Individual fish from each strain were injected intra-peritoneally with 0.1 ml Dexadresone (dexamethasone 2 mg ml^{-1}) corresponding to 200 µg dexamethasone per fish. Injected salmon were kept 48 h before the initiation of the infection.

Water: Fish and parasites were kept in a mixture of 50% dechlorinated municipal water and 50% de-ionized water with a final calcium carbonate hardness of 200 mg l^{-1}. Temperature was 13°C and pH 7.0. Water was continuously aerated and treated with an internal biofilter (Eheim) eliminating nitrite and ammonia.

Experimental design: The different fish groups were exposed together to infection with *Gyrodactylus salaris* in a 200 l aquarium. Thus, 5 untreated Conon and 10 untreated Lule river salmon were stocked with 5 dexamethasone treated Conon and 10 dexamethasone treated Lule salmon. Fish were differentiated by finclipping. Infection was achieved by cohabitation with 6 Conon salmon infected with a total of 386 parasites. Parasites from the infected donor fish were allowed to spread to the experimental fish during the following experimental period.

Parasite counting: The number of parasites present on various body parts and fins of MS222- (50 mg l^{-1}) anaesthetized salmon were counted under the dissection microscope (7 to 40× magnification) according to Buchmann & Uldal (1997). Parasites were counted once a week.

Mucous cell counting: At the end of the experiment, 8 wk post-infection, tail fins from fish in the various groups including uninfected controls were formalin-fixed and stained with 1% Alcian Blue in 3% acetic acid (Buchmann & Uldal 1997). Following mounting in Aquamount, the number of superficial mucous cells were counted (blinded) in a light microscope on an area of 0.75 mm^2 (3 zones of 0.25^2 mm caudal-anterior direction) on each fin (200× magnification).

Statistics: Infection levels were expressed as prevalence (percentage of hosts infected) and mean intensity (mean number of parasites per infected fish) according to Bush et al. (1997). Differences of means between groups were evaluated with the Tukey test with a probability level of 0.05.

Results and discussion. All fish became infected and kept the parasites throughout the experimental period of 8 wk. The *Gyrodactylus salaris* population increased significantly more on Conon salmon (mean intensity 98 parasites per fish in Week 8) compared to Lule salmon (mean intensity 35 parasites per fish). In addition, the dexamethasone treated Scottish salmon experienced an even higher infection level within the period (mean intensity 280 parasites per fish). In contrast, corticosteroid treatment of Lule salmon resulted in only slightly larger parasite populations compared to untreated Lule river salmon. Thus, treated Lule salmon harboured a mean of 40, 36 and 33 parasites in Weeks 6, 7 and 8, respectively, compared to 32, 22 and 35 parasites in untreated fish (Table 1). Some mortality was seen during the experiment (Table 1). Mucous cell densities varied between groups. In Week 8, untreated and uninfected Conon salmon had a higher number of mucous cells per unit area compared to corresponding Lule salmon. When untreated fish became infected, the mucous cell density decreased markedly within 8 wk (both salmon strains). This was also seen in dexamethasone-treated Lule salmon but not in treated Conon salmon, which did not show a decrease of mucous cell density following infection (Table 2).

The present investigation indicated that the Baltic Lule strain is more resistant, compared to the Scottish Conon salmon, to infection with the Norwegian Lærdalselva strain of *Gyrodactylus salaris*. This is in line with the notion that the Baltic salmon has co-existed with this monogenean for an extended time period whereby a relative resistance of the host could

Table 1. Mean intensity (MI) standard deviation (±SD) and mortality (M%) of salmon groups during the 8 wk infection experiment. A, B, C, D, E, G: significantly different ($p < 0.05$) in relation to corticosteroid treated Conon salmon. F: significantly different ($p < 0.05$) in relation to untreated Conon salmon

	Week							
	0	1	3	4	5	6	7	8
Conon salmon untreated								
MI	0	3.8	17.2	22.4	27.0	59.5	71.0	98.0
(SD)	(0)	(1.5)	(4.1)	(5.8) B	(10.1) C	(16.7) D	(32.0) E	(34.6) G
M%	0	0	0	0	20	20	40	40
Conon salmon treated								
MI	0	5.7	31.3	47.0	56.5	162.0	128.7	279.7
(SD)	(0)	(4.2)	(17.5)	(10.4)	(8.6)	(40.6)	(14.2)	(65.5)
M%	0	20	20	20	20	20	40	40
Lule salmon untreated								
MI	0	4.1	9.8	17.0	19.7	32.7	22.4	35.3
(SD)	(0)	(3.8)	(5.3) A	(11.5) B	(10.5) C	(20.9) D	(15.2) E, F	(31.8) G
M%	0	0	10	10	10	10	10	20
Lule salmon treated								
MI	0	3.0	21.5	21.9	27.6	39.9	36.1	32.8
(SD)	(0)	(1.4)	(9.2)	(9.6) B	(11.6) C	(19.8) D	(20.7) E	(17. 4) G
M%	0	10	10	10	20	20	30	40

Table 2. Mucous cell number (mean ±SD) on 0.75 mm caudal fin from different salmon groups. A: significantly different ($p < 0.05$) in relation to untreated and uninfected Conon salmon. B: significantly different ($p < 0.05$) in relation to treated and infected Conon salmon

	No. of fish	Mucous cell no. (mean ±SD)
Conon salmon Untreated and uninfected	4	610 (109)
Lule salmon Untreated and uninfected	4	376 (74)
Conon salmon Untreated and infected	3	419 (141)
Lule salmon Untreated and infected	4	217 (68) A, B
Conon salmon Treated and infected	2	608 (207)
Lule salmon Treated and infected	3	189 (102) A, B

have been selected (Bakke et al. 2002). In contrast, the Scottish salmon has never been exposed to this pathogen and has not evolved resistance. The susceptibility of the Scottish salmon is in accordance with previous results obtained by Bakke & MacKenzie (1993). No information about the Lule salmon susceptibility has been presented previously. However, our results point to a relative resistance which can be compared with other Baltic salmon strains. The Neva strain has been shown to respond after less than 30 d post-infection at a peak infection intensity of less than 150 parasites; the other tested Baltic salmon from the Indals river responded within 40 d (Bakke et al. 2002). Lule salmon did not show higher intensities than this but the low peak occurred after 6 wk, which is delayed compared to the other Baltic salmon. The mechanisms involved in resistance of salmonids against infections with gyrodactylids are not clearly described (Buchmann & Lindenstrøm 2002). However, a number of studies have indicated that the fish immune responses play a role. Thus, Gyrodactylid infection elicits cytokine expression in fish skin (Lindenstrøm et al. 2003) whereby a number of immunological reactions are initiated. It has also been described that complement-factors from salmonids bind to and kill gyrodactylids (Buchmann 1998, Harris et al. 1998), and the level of complement-factor expression during infection is increased in Neva salmon compared to Norwegian salmon (Bakke et al. 2002). Furthermore, host macrophages colonize and kill gyrodactylids *in vitro* (Buchmann & Bresciani 1999) and dexamethasone significantly down-regulates macrophage activity (Pagniello et al. 2002). In addition, other immunosuppressants, such as hydrocortisone (Harris et al. 2000), have been shown to inhibit the host resistance towards infection with specific gyrodactylids, which further suggests the role of immune factors in host specificity. However, the effects of corticosteroids on mammals are quite complex (Wilckens & De Rijk 1997) and it cannot be excluded that other mechanisms in fish might be affected by the steroid treatment.

Mucous cell densities have previously been suggested to influence behaviour and survival of gyrodactylids on rainbow trout skin (Buchmann & Bresciani 1998). It was indicated that parasites select mucous cell rich areas during initial colonization but escape these spots during the host response phase. In the present experiment, the most susceptible salmon (Conon) showed a higher density of mucous cells than Lule salmon (Table 2). When infected for 8 wk, both salmon races exhibited a decrease of mucous cell densities of their tail fins. Furthermore, dexamethasone treatment seemed to inhibit the mucous cell discharge of infected Conon salmon concomitant with an extensive parasite colonization. This was not seen in Lule salmon, which only permitted a low parasitization. These observations justify further studies on the role of mucous cell dynamics in host response and host specificity.

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