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Increased susceptibility of Atlantic salmon *Salmo salar* to infections with *Gyrodactylus derjavini* induced by dexamethasone bath treatment

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Abstract

Dexamethasone, a known immunosuppressant, was administered by bath or injection to Atlantic salmon *Salmo salar* (Conon stock) to study if this treatment could affect the susceptibility of fish to infections with a Danish strain of *Gyrodactylus derjavini* (Monogenea). Three groups of *S. salar* (Conon stock) were immersion treated either with 10, 60 or 240 μg dexamethasone l^{-1} water, respectively. In addition, one group (positive control) was treated intraperitoneally with 200 μg dexamethasone per fish and one negative control group was kept untreated. A single *G. derjavini* parasite was placed on the anal fin of each fish and the infection was subsequently monitored weekly for 6 weeks. An increase in parasite populations on the salmon was positively correlated with the amount of immunosuppressant used. Infection levels in the group immersion treated with dexamethasone (240 $\mu g\,l^{-1}$ water) and in the i.p. treated positive control group were significantly higher compared to the untreated control group.

Introduction

Members of the genus *Gyrodactylus* (Monogenea) are ectoparasitic platyhelminths and some species are serious pathogens (Bakke & MacKenzie, 1993). Thus, *Gyrodactylus salaris* has been associated with high mortalities in Norwegian Atlantic salmon populations (Harris *et al.*, 2000) and *Gyrodactylus derjavini* has caused problems in rainbow trout and brown trout, whereas salmon is considered to be relatively resistant to *G. derjavini* infections (Buchmann & Uldal, 1997; Bakke *et al.*, 1999). These parasitic worms are viviparous and multiply relatively fast on susceptible hosts. Increased susceptibility of fish to infections after administration of immunosuppressive agents has primarily been investigated with respect to bacterial, fungal and protozoan diseases (Anderson *et al.*, 1982; Pickering & Duston, 1983; Houghton & Matthews, 1990). In contrast, there have been relatively few studies that have investigated the interaction between helminths and immunologically suppressed fish hosts. The susceptibility of salmonids to *G. salaris* was increased following hydrocortisone treatment (Harris *et al.*, 2000). Intraperitoneal administration of either testosterone (Buchmann, 1997) or dexamethasone (Lindenstrøm & Buchmann, 1998) to rainbow trout fry (*Oncorhynchus mykiss*) seems to decrease the host response against *G. derjavini*. The administration of steroid hormones to salmonids by immersion is possible (Baker *et al.*, 1988), and in the present work the effect of dexamethasone treatment (immersion and injection) on the susceptibility of Atlantic salmon, *Salmo salar*, to infections with *G. derjavini* is described.

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Materials and methods

Salmo salar

Hatchery reared salmon *S. salar* (Conon stock) fry were obtained from the Danish Centre for Wild Salmon, Brusgaard Fisheries (Jutland) and kept in 180 l aquaria in

the laboratory. The naïve fish were fed ad libitum (4 weeks) up to a weight of 0.9–1.3 g (length 4.2–5.9 cm). The salmon were kept at a water temperature of 11–12°C with a 12:12 h light-darkness cycle prior to and during experimentation.

Gyrodactylus derjavini

A monoculture of *G. derjavini* parasites was established from collections at a Danish fish farm (Uggerby stream system in Northern Jutland) and subsequently maintained on rainbow trout in the laboratory.

Corticosteroid treatment

Fish were exposed to the synthetic corticosteroid dexamethasone (fluoro-methyl-prednisolone) (Boehringer Ingelheim) in two ways: (i) immersion treatment by subjecting fish to 10 $\mu g\,l^{-1}$ (group A), 60 $\mu g\,l^{-1}$ (group B) or 240 $\mu g\,l^{-1}$ water (group C) for 48 h; or (ii) as intraperitoneal (i.p.) injection with 100 μl fish^{-1} (200 μg) (group D, positive control). Twenty naïve fish were used in each of the bath treated groups, 15 fish were injected i.p. and a control group of 15 fish was untreated. Both fish immersed and injected with dexamethasone were kept in freshwater 48 h prior to infection with parasites.

Infection procedure

Infections were conducted in every group by applying a single gravid *G. derjavini* parasite to the anal fin of each Conon salmon after anaesthetizing the fish in 50 $mg\,l^{-1}$ MS-222 (methane tricaine sulphonate, Sigma). Each infected fish was subsequently transferred to a separate 10 l aquarium with 3 l of freshwater. The water was changed partly (75%) every 3rd or 4th day. Infections were monitored microscopically (7–40 × magnification) on anaesthetized fish (MS-222, 50 $mg\,l^{-1}$) every 7th day.

Data processing

The abundance and prevalence of infection were calculated according to Margolis *et al.* (1982). The Mann-Whitney U-test was applied for the detection of differences in abundance ($P < 0.05$). Variance to mean ratios were also calculated.

Results

Prevalence

The prevalence of infection of *G. derjavini* in untreated and treated groups differed from week 1 post infection (p.i.) (fig. 1). Higher prevalences were found in treated groups A, B, C and D compared to the untreated control

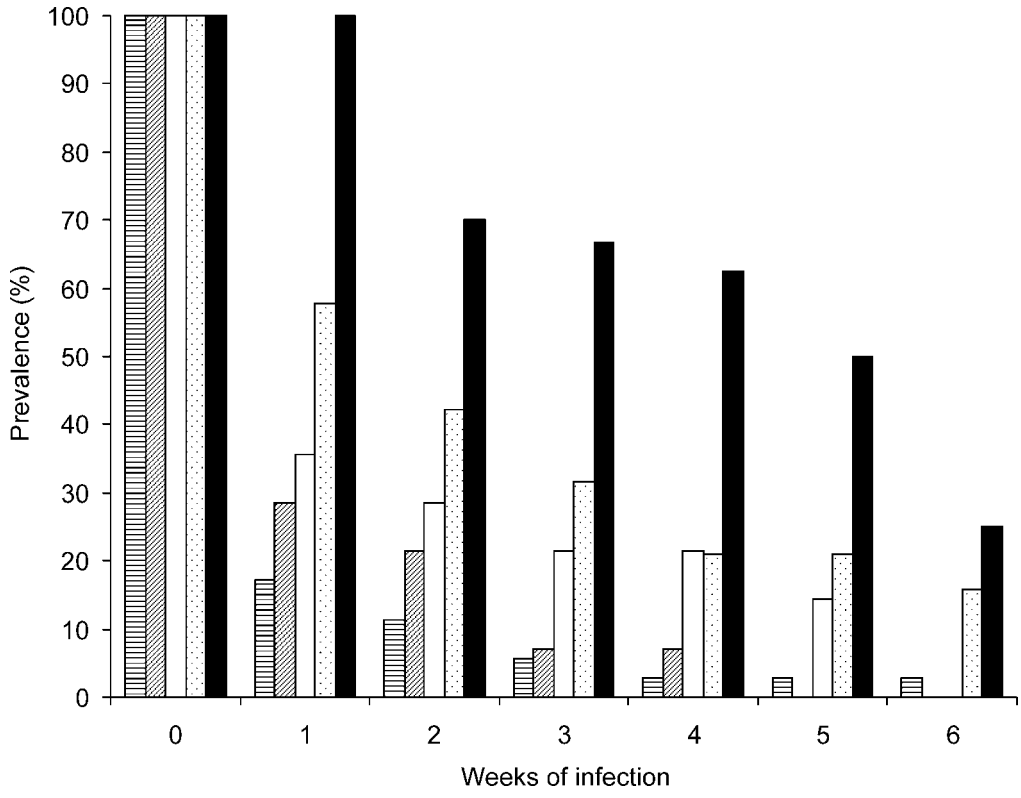

Fig. 1. The prevalence (%) of *Gyrodactylus derjavini* in five groups of Conon salmon (*Salmo salar*) during 6 weeks of infection. ▤, control, non-treated; ▧, group A, 10 μg dexamethasone l^{-1} water; □, group B, 60 μg dexamethasone l^{-1} water; ▨, group C, 240 μg dexamethasone l^{-1} water; and ■, group D, intra peritoneal injection with 200 μg fish^{-1}.

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group. Groups C and D had significantly higher prevalences all six weeks of the experiment.

Abundance

Significantly higher abundances of infection of *G. derjavini* were found in the dexamethasone immersion treated group C compared to the untreated control group during the first 3 weeks ($P < 0.05$) (fig. 2). The i.p.-treated group (group D) had a significantly higher abundance throughout the entire experimental period compared to the untreated control ($P < 0.05$). The abundances of groups A or B were only insignificantly higher during the experimental period compared to the untreated control group. An overdispersed distribution ($\mathrm{Var}/\ \bar{x} > 1$) of parasites was found in all groups during the whole experimental period (table 1). The ratio peaked at week 4 p.i. for group B (ratio = 10.48), at week 5 p.i. for group C (ratio = 12.21) and at week 6 p.i. for the injected group D (ratio = 20.44). During the experiments five fish died in the i.p.-treated group (at week 3 p.i.) and two fish in the untreated control group (at week 1 p.i.). Parasites on dead fish were excluded from the calculations of abundances.

Discussion

The results indicate a significant increase in the susceptibility of salmon to infections with *G. derjavini*, following immersion treatment with 240 μg dexamethasone l^{-1} water or after i.p. injection treatment with 200 μg $fish^{-1}$. Both data on prevalence and abundance of infection support this view. An overdispersed distribution of *G. derjavini* in the present study (table 1) is consistent with the distribution and heterogeneity in the course of the gyrodactylid infection reported by Bakke & MacKenzie (1993). Infection by cohabitation has previously been used in a number of studies on the susceptibility of salmonids towards gyrodactylids (Bakke & MacKenzie, 1993; Bakke *et al.*, 1999). Such an infection mode has also been used in investigations of host responses (Lindenstrøm & Buchmann, 2000) and experimental immunosuppression of salmonids by the corticosteroid dexamethasone (Lindenstrøm & Buchmann, 1998) and hydrocortisone (Harris *et al.*, 2000). This method is based on parasite exposure and the subsequent spread of infection from heavily infected donor fish kept with uninfected recipients. However, the experimental procedure described in the present paper is conducted in our laboratory for propagation of micropopulations of a certain species of *Gyrodactylus* and is also suitable for intensive studies on host susceptibility. Thus, the fecundity of single parasites can be monitored in this way (Cable *et al.*, 2000).

The low susceptibility of the untreated group of Conon salmon to *G. derjavini* (fig. 2) corresponds well with previous reports on this salmon stock (Buchmann & Uldal, 1997; Bakke *et al.*, 1999). Abundances of groups A and B were not significantly increased during the experimental period, which might be related to the low uptake of dexamethasone from water. The induction of susceptibility in Conon salmon towards *G. derjavini*, when

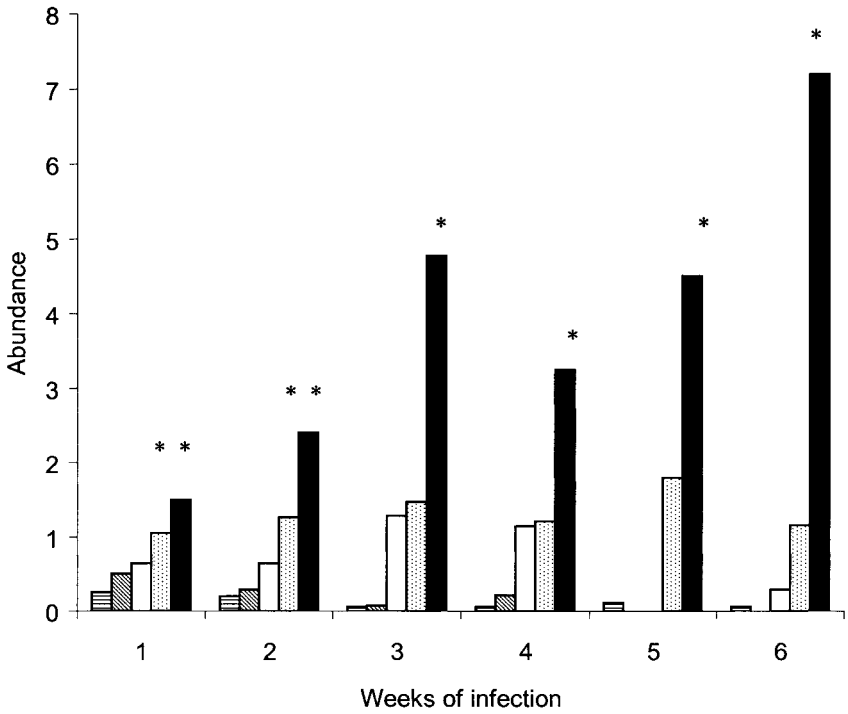

Fig. 2. The abundance (mean no. of parasites per fish) of *Gyrodactylus derjavini* in four dexamethasone treated groups and one control group of Conon salmon (*Salmo salar*) during 6 weeks post-infection (*$P < 0.05$). ▤, control, non-treated; ▧, group A, 10 μg dexamethasone l^{-1} water, □, group B, 60 μg dexamethasone l^{-1} water; ▨, group C, 240 μg dexamethasone l^{-1} water; and ■, group D, intraperitoneal injection with 200 μg $fish^{-1}$.

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Table 1. Variance to mean ratios (Var./ $\bar{x}$) for treated and untreated groups of Conon salmon.

	Weeks					
Group	1	2	3	4	5	6
Control	1.68	2	0.97	2	4	2
A	2.38	1.31	1	3	–	–
B	1.58	2.54	6.15	10.48	5.97	2.38
C	1.21	3.24	6.21	5.65	12.21	7.70
D	0.63	1.41	3.13	3.41	5.97	20.44

A, 10 μg dexamethasone l^{-1} water; B, 60 μg dexamethasone l^{-1} water; C, 240 μg dexamethasone l^{-1} water; and D, intraperitoneal injection with 200 μg $fish^{-1}$.

fish were treated with the highest dose rates of dexamethasone as in group C and D (240 $\mu g\, l^{-1}$ water and 200 μg $fish^{-1}$, respectively) could be due to immunosuppression of the fish. The injection procedure itself does not affect susceptibility of the host to infections with *G. derjavini* (Larsen *et al.*, 2002). The induction of salmonid susceptibility to gyrodactylid infections after corticosteroid administration has previously been documented by Lindenstrøm & Buchmann (1998) and Harris *et al.* (2000). Lindenstrøm & Buchmann (1998) showed that rainbow trout increase their susceptibility to *G. derjavini* infections following the administration of dexamethasone (30 $\mu g\, g^{-1}$ body weight). Harris *et al.* (2000) concluded that brown trout, *Salmo trutta* – normally relatively resistant to *G. salaris* – increase their susceptibility to the gyrodactylid infection after the administration of hydrocortisone acetate (approx. 130 $\mu g\, g^{-1}$ body weight).

Although it cannot be excluded that dexamethasone influences a number of non-immunological factors, its immunosuppressive effect is well known (Lindenstrøm & Buchmann, 1998), suggesting that the increased susceptibility found in the present study may have an immunological basis. This could further indicate that the specificity of fish–parasite systems is influenced by the fish immune system.

Acknowledgements

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