Vol. 55: 23–30, 2003 | DISEASES OF AQUATIC ORGANISMS
Dis Aquat Org | Published June 20

Immunization of rainbow trout *Oncorhynchus mykiss* against *Discocotyle sagittata* (Monogenea)

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ABSTRACT: Rainbow trout *Oncorhynchus mykiss* were injected intraperitoneally with 2 different *Discocotyle sagittata* extracts dissolved in PBS and subsequently exposed to controlled infection. Immunization resulted in significantly reduced ($p < 0.0001$) worm intensities in >50% of vaccinated fish (response arbitrarily defined as parasite burdens < mean control intensity – 1 SD), irrespective of the immunization regime (different parasite extracts, dosing and application schedules) employed. The protective effect of worm extract applied in Freund's complete adjuvant (FCA) did not differ significantly from extract given in PBS. Vaccination with embryonated parasite eggs extract and with FCA alone did not result in partial immunity, suggesting the observed protective effect is specific. Immunized fish had significantly higher specific antibody titres at the time of dissection (as determined by ELISA) than both naïve and control fish. Overall, a significant negative correlation was found between antibody titres and worm burdens, suggesting immunoglobulins are implicated in mediating partial immunity. Western blot tests indicated the 2 different worm extracts used to immunize fish share antigens, but each one primarily induced recognition of a distinct band (30 and 38 kDa). Immunization seems to promote a shift between 2 equilibria, rather than progressively increasing protection. This would explain why boosting did not increase immunity, and why 2 different extracts primarily inducing recognition of 2 distinct antigens provide similar degrees of protection. Although several other non-specific and cellular factors are likely to be involved in controlling parasite numbers, it cannot be excluded that antibodies could be involved in mediating the observed partial immunity.

KEY WORDS: *Oncorhynchus mykiss* · Trout · Monogenea · *Discocotyle sagittata* · Immunization · Antibodies

INTRODUCTION

Fish in the wild are rarely infected with large numbers of parasites; this could be a consequence of a well developed host immune system (Woo 1992, Secombes & Chappell 1996), in addition to the stochastic factors commonly invoked to explain naturally overdispersed parasite distributions. However, under the stressful conditions experienced by fish in intensive farms, parasitic infections leading to significant pathology and mortality do occur (Thoney & Hargis 1991, Woo 1992). Relatively little is known about piscine innate immunity to helminths, and the immune mechanisms underlying the documented cases of host responses to parasitic infections have been insufficiently described (Buchmann 1999, Buchmann & Lindenstrøm 2002). However, as reviewed by Buchmann et al. (2001), several investigations have demonstrated that teleosts can develop partial protective immunity against ectoparasitic monogeneans after natural infection (Jahn & Kuhn 1932, Nigrelli & Breder 1934, Nigrelli 1937), experimental challenges (Scott & Robinson 1984, Scott 1985, Slotved & Buchmann 1993, Bondad-Reantaso et al. 1995) and active immunization (Vladimirov 1971, Kim et al. 2000).

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Discocotyle sagittata is a monogenean parasite of freshwater salmonid fishes which can be an important pathogen of farmed trout (Gannicott 1997). Previous studies (Gannicott 1997, Gannicott & Tinsley 1997, 1998a,b) described the principal biological characteristics of the parasite; these provided the basis of a dependable experimental infection system which allowed controlled studies of early stages of the host–parasite interaction (Rubio-Godoy & Tinsley 2002). In the present study, fish were injected with crude parasite extracts prior to *D. sagittata* challenge to assess whether the attempted vaccination altered the outcome of controlled infections.

MATERIALS AND METHODS

Fish. *Oncorhynchus mykiss* fingerlings were purchased from a parasite-free hatchery. Batches of 30 to 50 fish were maintained in 500 l tanks at 13°C and fed 3.0 mm commercial feed pellets (Trout floating 30; BOCM Pauls). Animals were allowed at least 7 d to recover from transport-related stress before the experimental procedures.

Parasites. Rainbow trout naturally infected with *Discocotyle sagittata* from the Isle of Man were transported to Bristol and housed at 13°C under a light:dark regimen of 12:12 h. Parasite eggs were collected by filtration (125 µm nylon mesh sieve), washed in distilled water and incubated at 13°C under a 12:12 h light:dark photoperiod. After embryonation (ca. 28 d), egg batches hatch *en masse* in the first hour of the dark period (Gannicott & Tinsley 1997), allowing collection of recently emerged infective stages. Under the dissecting microscope, batches of 100 active oncomiracidia were collected with a Pasteur pipette and placed in Petri dishes; they were never allowed to reach an age >2 h before use.

Infection procedure. Fish (ca. 20 cm long) were placed individually in infection tanks (ca. 11.5 l, 13°C) and exposed to 100 active oncomiracidia for 24 h, the first 12 h of which were in darkness. Afterwards, they were kept in 500 l tanks at 13°C for 1 mo. Experimentally infected fish were anaesthetised terminally with 0.05% MS222, bled by caudal vein puncture to determine antibody titres, weighed and measured. Gills were removed and examined under the dissecting microscope, and the number of parasites per gill arch was recorded. Parasites were grouped in different (categorical) developmental cohorts based on the number of pairs of clamps they had grown (Gannicott & Tinsley 1997, 1998a,b). Age cohort data were transformed into continuous variables by calculating the mean number of pairs of clamps attained by worms recovered from individual fish (Rubio-Godoy & Tinsley 2002). This average, the Developmental Index, was calculated as follows:

$$\text{Developmental Index} = \frac{\sum(\text{worms recovered} \times \text{developmental cohort})}{\text{total number of worms}}$$

Blood samples were allowed to clot overnight (4°C) and sera were kept at –70°C until analysed.

Immunization. A crude worm extract was prepared by adapting a method developed for *Taenia* spp. (Molinari et al. 1993). Briefly, whole frozen and formalin-fixed worms were washed and homogenised in PBS pH 7.0. The homogenate was centrifuged (30 min, 3400 × *g*) and the supernatant filtered through 0.2 µm Millipore membranes; this filtrate was subsequently called 'worm extract'. The centrifugation pellet was retained and further processed in parallel to the filtrate; this was subsequently referred to as 'pellet extract'. Both extracts were dialysed against PBS and lyophilised in sterile vials. The parasite 'egg extract' was prepared by homogenising embryonated eggs incubated for 2 wk at 13°C (about halfway through embryo development) in PBS pH 7.0, and filtration through 0.2 µm Millipore membranes. The antigenic preparations were kept at –70°C until used and their total protein concentration was determined by the method of Lowry et al. (1951).

Anaesthetised fish were treated with 10 µg (total protein concentration) each of worm, pellet or egg extract dissolved in 0.5 ml PBS pH 7.0 by intraperitoneal (i.p.) injection, without adjuvant. Groups immunized once were injected 2 wk before parasite challenge; those vaccinated twice were treated 4 and 2 wk or 6 and 4 wk prior to exposure. Control groups received i.p. PBS only and were infected during the same week's post-injection as immunized fish. One further group received 10 µg pellet extract i.p. in 0.25 ml PBS emulsified in an equal amount of Freund's complete adjuvant (FCA; Sigma Chemical) 4 wk prior to infection; controls for this group received i.p. FCA only and were challenged concurrently.

Immunological parameters. Ethanol-fixed *Discocotyle sagittata* were sonicated on ice without the addition of anti-proteases (Ultrasonic Liquid Processor XL 2020; Heat System) and used as antigenic source. ELISA was performed in 96-well microtitre plates coated (12 h at 5°C) with antigen (5 µg ml^{-1} protein) dissolved in 100 µl coating buffer (4.29 g $Na_2CO_3 \cdot 10H_2O$ and 2.93 g $NaHCO_3$ in 1 l of distilled water, pH 9.6). Microtitre plates were then washed 5 times with washing buffer (PBS with 0.05% Tween-20; Merck). Uncoated sites were blocked by adding 100 µl blocking buffer (PBS with 0.5% bovine serum albumin, BSA; Sigma Chemical) per well for 15 min. Between the following steps, plates were washed 5 times with washing buffer. Sera were diluted serially

(1:2 to 1:1024) in dilution buffer (PBS with 0.1% BSA) and 100 µl of each dilution was added in duplicate to each of the coated wells and incubated for 1 h under gentle agitation. Rabbit anti-salmon Ig (Buchmann & Pedersen 1994) (100 µl at 1:1000 in dilution buffer) was added to each well and incubated for 1 h. Finally, 100 µl peroxidase-conjugated goat anti-rabbit Ig (1:2000 in dilution buffer; Sigma Chemical) was supplied to each well. Following incubation for 1 h, 100 µl enzyme substrate (*o*-phenylenediamine, OPD; Sigma Chemical) was added per well, and plates incubated for 10 to 15 min. Enzymatic reaction was stopped by adding 50 µl 3M HCl per well, and optical density (O.D.) determined at 492 nm using an ELISA-reader (Multiscan RC, Type 351; Labsystems). Each plate included 2 controls: wells with all antibodies and substrates except sample material (primary antibodies) as negative controls (blanks), and sera obtained from naïve fish. Values greater than 2 times the (blank) background absorbance were considered to be positive. ELISA was validated by means of the O.D. readings obtained from naïve sera, which were the same in the different plates/assay lots.

Western blots. Electrophoresis was done using a commercially available electrophoresis system (NuPAGE; Novex). Proteins from *Discocotyle sagittata* were separated by a 10% polyacrylamide gel at neutral pH by applying 25 µg total protein of the sonicated antigen in sample buffer to each well. All samples were run under non-reducing conditions using MOPS (3-[N-morpholino] propane sulfonic acid) running buffer (200 V for 1 h). Antigens run on SDS-PAGE were transferred to 0.45 µm nitrocellulose membranes using a blotting module (Xcell 50II Mini-Cell Blot module; Novex) (30 V for 1 h). The nitrocellulose membrane was then blocked for unspecific binding with blocking buffer (PBS pH 7.2 with 1% skimmed milk powder) for 15 min after which the trout antiserum dilution (1:20) was added. Following incubation for 1 h the membrane was washed 3 × 5 min with washing buffer (PBS with 0.05% Tween-20) before addition of rabbit anti-salmon Ig (1:1000). After incubation for 1 h and the 3 × 5 min washes, horseradish peroxidase conjugated goat-anti-rabbit antibody (Sigma A-0545) (1:2000) was added to the membranes and incubated for 1 h. After 3 × 5 min of washing, the blot was developed using diamino-benzidine-tetrahydrochloride (DAB) (Sigma D-5905) in PBS with H_2O_2.

Statistical analysis. Data were analysed with the statistical package SPSS for Windows 10.0. One-way ANOVA (General Linear Models) was used to analyse the effect of immunization on numbers of worms recovered and on antibody titres in each treatment group, and to validate ELISA tests; the significance level was set at $p < 0.05$. Tukey's test was used for post-hoc analysis. For each experimental group, the mean intensity (Bush et al. 1997) was calculated. As an arbitrary measure of efficacy, the percentage of the immunized population harbouring fewer parasites than the overall mean control intensity minus 1 SD was calculated; efficacy rates are only illustrative and were not used in any statistical calculations.

RESULTS

Fish measured 19.6 ± 0.23 cm (mean ± SE) in length and weighed 96.6 ± 3.24 g; there was no significant difference between experimental (n = 118) and control (n = 104) fish (length $F_1 = 0.614$, $p = 0.434$; weight $F_1 = 0.414$, $p = 0.521$). In all trials, fish were dissected 1 mo p.i. and all experimentally infected animals harboured parasites. Although worm numbers differed between treatment groups (see below), the Developmental Index calculated for each did not: 1 mo p.i. an overall mean of 1.53 ± 0.07 was found, which did not differ significantly between groups ($F_1 = 0.134$, $p = 0.715$).

Treatment with 1 or 2 doses of 10 µg worm or pellet extract applied in PBS resulted in most cases in significantly reduced worm burdens in immunized as compared with control fish. Table 1 shows the mean parasite intensities found in control and immunized groups, as well as the statistical probability and the illustrative, arbitrary efficacy rate calculated for each trial. As per definition, immunization was effective in ≥50% of fish receiving worm or pellet extract, since they exhibited intensities equal to or less than 37 worms per fish; overall, 16% of control fish had burdens in this range. In all but 1 trial (pellet, 2 wk), statistical analysis demonstrated that the protective effect of immunization suggested by the arbitrary efficacy rate was significant. Parasite intensities in egg extract-injected fish were not different from their respective controls. The degree of protection conferred by immunization did not increase with different application regimes nor boosting: despite differences in the arbitrarily calculated vaccination efficacies, mean intensities in immunized fish are comparable throughout (Table 1; post-hoc analysis showed no significant differences between groups).

Overall, worm extract immunization significantly reduced infection levels ($F_1 = 33.79$; $p < 0.0001$), but neither the immunization regime (1 or 2 doses; different application schedules) ($F_2 = 0.381$; $p = 0.685$) nor the interaction between regime and application of extract ($F_2 = 0.765$; $p = 0.469$) significantly modified the result. A similar situation was found when pellet extract was applied in PBS: immunization significantly modified the infection outcome ($F_1 = 22.19$; $p < 0.0001$) while neither regime ($F_2 = 2.77$; $p = 0.069$), nor the regime × immunization interaction ($F_2 = 1.61$; $p =$

Table 1. Effect of *Discocotyle sagittata* extract immunization on parasite recovery 1 mo post-infection. FCA: Freund's complete adjuvant. Efficacy: percentage (and fraction) of immunized population harbouring fewer worms than overall control mean –1 SD, i.e. intensities equal to or less than 37 worms/host. ns: not significant

Worm			Pellet			Egg		Pellet in FCA
2 wk	4–2 wk	6–4 wk	2 wk	4–2 wk	6–4 wk	2 wk	6–4 wk	4 wk
Efficacy								
67% (6/9)	50% (9/18)	67% (8/12)	50% (6/12)	75% (15/20)	82% (9/11)	0% (0/10)	0% (0/10)	56% (9/16)
Control group; mean worms/host ± SE (n)[a]								
45.9 ± 6.28 (10)	45.3 ± 1.49 (17)	46.2 ± 2.98 (10)	42.4 ± 2.93 (9)	42.4 ± 1.18 (19)	47.38 ± 4.65 (8)	41.6 ± 4.25 (8)	39.3 ± 1.33 (7)	43.4 ± 2.97 (16)
Immunized group; mean worms/host ± SE (n)								
32.0 ± 3.19 (9)	36.9 ± 2.13 (18)	34.9 ± 2.32 (12)	37.6 ± 4.12 (12)	31.1 ± 1.89 (20)	29.0 ± 3.92 (11)	38.0 ± 2.76 (10)	40.5 ± 3.44 (10)	36.2 ± 3.47 (16)
p								
0.001	0.003	0.007	ns	<0.0001	0.008	ns	ns	0.026

[a]Note: Control groups received intraperitoneal (i.p.) PBS only and were infected during the same weeks post-injection as immunized fish, except for the pellet in FCA 4 wk control group, which received i.p. FCA

0.206), significantly affected the results. The application of pellet extract in Freund's complete adjuvant (FCA) 1 mo prior to infection resulted in significant partial protection in >50% of immunized fish (Table 1). However, the results obtained with adjuvanted pellet extract were comparable to those from fish that received 1 dose of pellet extract in PBS ($F_1 = 0.068$; p = 0.797). Control fish that received FCA and no extract likewise had similar parasite intensities to control fish receiving saline only ($F_1 = 0.048$; p = 0.829).

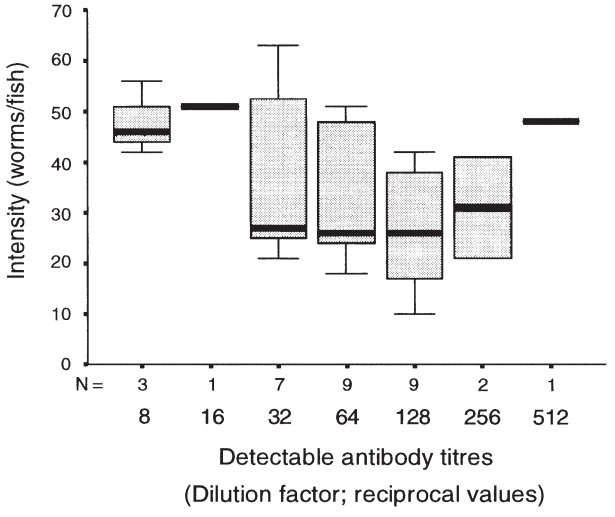

Fig. 1. Correlation between *Discocotyle sagittata* intensity and antibody titres at the time of rainbow trout *Oncorhynchus mykiss* dissection. Figure illustrates the median (thick line), the interquartile range containing 50% of the values (bar) and the range (whiskers) for each dilution factor

ELISA was used to determine the mean anti-*Discocotyle sagittata* antibody titres at the time of dissection of experimentally infected and naïve fish, which corresponds to 6 wk post injection for fish immunized once, and 8 to 10 wk post first-injection for those immunized twice. Titres were expressed as the 2-fold dilution factor beyond which no response was detected. Eight control and 16 immunized serum samples from each worm and pellet in PBS extract-injected groups were selected at random for immunological analysis. Naïve serum gave positive responses up to a mean dilution factor (±SE) of 3.71 ± 0.34, control serum of 5.06 ± 0.14 and immunized serum of 6.00 ± 0.24; statistical analysis validated the ELISA by showing the naïve serum O.D. readings in all plates were comparable, indicating that immunization significantly modified detectable antibody titres ($F_2 = 8.601$; $p < 0.0001$), and post-hoc tests showed the 3 groups were significantly different. Moreover, a significant negative correlation was found between the number of parasites recovered from immunized fish (pooled worm- and pellet-extract immunized groups) and antibody titres, expressed as 2-fold dilution factors ($R^2 = 0.866$; $F_6 = 26.997$; $p < 0.0001$; Fig. 1).

Western blot results indicated extract-immunized fish recognise 6 to 8 antigenic bands with different molecular sizes (Fig. 2): worm extract, ca. 100, 50, 45, 40, 38, 30, 20 and 11 kDa; pellet extract, ca. 126, 50, 30, 18, 16 and 4 kDa. There is however a clear difference in recognition intensity: pellet extract–immunized sera reacted mainly against a ca. 30 kDa band, while worm extract-immunized sera primarily recognised antigens ca. 38 kDa in size. Sera from infected control fish also reacted faintly against antigenic bands ca. 100, 50, 38, 30 and 4 kDa in size.

DISCUSSION

The present experiments demonstrate that active immunization of rainbow trout with *Discocotyle sagittata* extracts confers partial protection against infection. As reviewed by Buchmann et al. (Buchmann 1999, Buchmann et al. 2001, Buchmann & Lindenstrøm 2002), a number of investigations have shown that teleost fishes are capable of mounting protective immune responses to monogenean parasites, which suggests that vaccination is possible. However, successful immunization has been achieved primarily against some bacterial and viral diseases (Nakanishi & Ototake 1997, Palm et al. 1998), and effective vaccines against helminths have proved mainly elusive. For instance, no protective immunity resulted from the injection of fish with sonicated *Neobenedenia girellae* (Bondad-Reantaso et al. 1995), nor with whole *Gyrodactylus alexanderi* (Lester 1974). However, partial protection was achieved in carp following immunization with dactylogyrids (Vladimirov 1971), and in rockfish *Sebastes schlegeli* vaccinated against the gill-inhabiting monogenean *Microcotyle sebastis* (Kim et al. 2000). The availability of a dependable experimental infection system with the polyopisthocotylean *D. sagittata* prompted the assessment of the role of immunity in controlling parasite burdens. Experimental exposure of rainbow trout to *D. sagittata* resulted in 100% prevalence and ca. 50% infection efficiency up to 3 mo p.i. (Rubio-Godoy & Tinsley 2002). This is in contrast with the usually low prevalence and intensity characteristics of natural monogenean infections. However, experimental, 3 mo old *D. sagittata* infection may represent only the early stages of the host-parasite interaction, before immunity controls worm burdens effectively. Long-term field- and lab-based studies with polystomatid monogeneans would support this hypothesis: in both scenarios, very efficient transmission rates lead to high intensities of juvenile stages, but parasite numbers undergo a marked attrition between invasion and maturity (Tinsley & Jackson 2002). Host immunity has been proposed to play a major role in regulating invading polystomatid juvenile numbers before they can contribute to transmission (Tinsley 1995, 1999, Tinsley & Jackson 2002). In fish-monogenean interactions, both non-specific and specific immune mechanisms have been proposed to protect fish against pathogens. Non-specific responses have been implicated in the control of gyrodactylid monogeneans, particularly complement (Buchmann 1998, Harris et al. 1998) and leucocytes (Buchmann & Bresciani 1999). Antibodies and various other immune compounds in rainbow trout mucus have been reported on several occasions (St Louis-Cormier et al. 1984, Buchmann & Bresciani 1998) and these could be involved in host defences (Buchmann 1999, Buchmann & Lindenstrøm 2002). *G. derjavini* distribution on rainbow trout exhibits a significant negative correlation with superficial mucous cell density, suggesting mucus may have an important effect on parasite site selection and infection dynamics (Buchmann & Bresciani 1998).

The present work suggests immunization against *Discocotyle sagittata* is feasible and that specific antibodies are 1 of the several possible factors controlling parasite numbers, since partial protection correlated significantly with increased serum immuno-globulin titres as determined at the time of dissection. However, no simple cause–effect relationship can be proposed for several reasons. First, no serum samples were obtained at the time of infection nor prior to dissection, therefore no direct inference can be made of their putative protective role during invasion and the early stages of parasite establishment. Moreover, it is uncertain if antibody levels at the time of infection were relevant in the groups immunized once, since only 2 wk had elapsed prior to challenge; but the possibility exists that antibodies are detected as soon as 10 d post-immunization in rainbow trout kept at 14 to 16°C (Palm et al. 1998). Second, no tests were performed to detect immunoglobulin (nor any other immune components) in mucus, which might be another route through which this gill-inhabiting parasite could be exposed to immune factors. Third, it is unlikely that immunoglob-

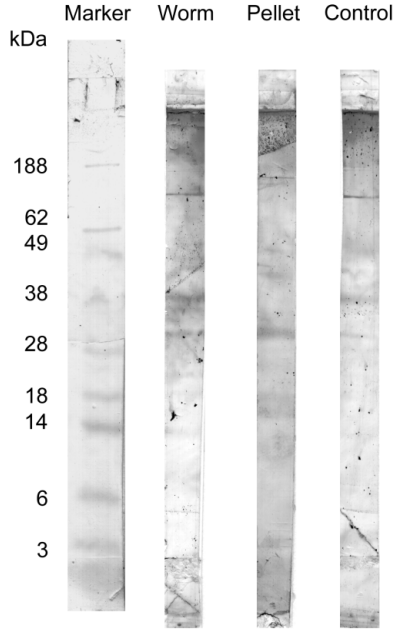

Fig. 2. Western blots of *Discocotyle sagittata* antigens revealed with sera from worm and pellet-extract immunized, and infected control rainbow trout *Oncorhynchus mykiss*. Sera were obtained at the time of dissection; i.e. at least 6 wk post-immunization with parasite extracts

ulin by itself is able to eliminate parasites, hence the lack of sterile immunity observed even in fish with relatively high antibody titres. And finally, the injection of parasite materials is likely to elicit inflammatory reactions, which could account for the reduced burdens recorded. Nevertheless, the specific, probably non-inflammatory nature of the non-sterile immunity observed is suggested by the fact that neither egg extract nor FCA alone modified infection outcome significantly. While it is uncertain whether embryonated eggs contain effective immunogens, and therefore induce non-specific responses, FCA is not only an immunization adjuvant but is itself a powerful elicitor of non-specific immunity (Anderson 1992, Secombes 1994).

As reported previously (Rubio-Godoy & Tinsley 2002), parasite intensity 1 mo p.i. in control fish was comparable in all groups, and reflected an infection efficiency of ca. 45%. Interestingly, mean intensities in immunized fish were similar irrespective of the distinct vaccination schemes applied to different experimental groups. Thus, dissimilar immunization regimes resulted in comparable degrees of protection, both in terms of the proportion of the population showing reduced worm burdens and in the mean intensities recorded. This suggests boosting does not apparently result in increased immunity. The comparable effect of worm- and pellet-extract immunization is striking considering the 2 parasite extracts primarily induced recognition of different antigens (Fig. 2). Overall, immunization seemed to induce the transition between 2 equilibria, rather than progressively increase protection and correspondingly decrease parasite burdens. As shown in Fig. 1, fish with antibody titres of 2^4 (dilution factor 16) or less (including naïve fish not depicted in Fig. 1) have mean (and median) parasite intensities consistently around 45 worms per host, which could be considered a 'permissive equilibrium parasite intensity'. In contrast, past an antibody titre of 2^5 (dilution factor 32) mean intensities abruptly fall to ca. 34 worms per host (and slightly lower medians), which could be described as a distinct 'immune equilibrium parasite intensity'. These 2 hypothetical states would be analogous to a separation of the immunized population into responding and non-responding individuals, as typically subdivided in clinical trials. In this study, 64% of fish could be considered to have responded to immunization, based on the arbitrary definition of efficacy relating to infection levels. Responder fish had higher mean antibody titres than both controls and immunized non-responders, but this difference was only significant for control fish (data not shown). Despite the non significant difference of antibody titres between responder and non-responder fish, it is interesting that elevated immunoglobulin levels tended to correlate with increased protection. In particular, fish with antibody titres >1:32 consistently harboured reduced parasite burdens, and these constituted the bulk of the immunized population irrespective of the antigenic extract employed (Fig. 1). Thus, it would be reasonable to argue that immunization shifted the balance between the 2 parasite intensities (and hypothetical equilibria) observed, suggesting that host responses are among the several possible factors which ultimately result in an overdispersed distribution. The occurrence of 2 distinct equilibria in this report would be analogous to *Discocotyle sagittata* infection levels found in field studies, in which a proportion of fish exposed to long-term transmission exhibit very high intensities, while others raised in the same conditions (and ponds) present low burdens (Gannicott 1997).

The actual host factors limiting parasite numbers are not known, but given the negative correlation found in this study between antibody titres and worm burdens, it would be tempting to propose that immunoglobulin accounts for the partial protection observed. The evidence, however, is not clear-cut. Naïve fish exhibited detectable antibody titres against the sonicated parasite; this positive response may be due to naturally occurring antibodies with low affinity to *Discocotyle sagittata*. Comparable antibody titres in control and immunized fish suggest that infection elicits a certain degree of humoral immunity, detectable 1 mo p.i. Moreover, naturally infected fish exhibit very high anti-*D. sagittata* antibody levels (author's unpubl. result), which do not seem to confer effective protection against either established or invading parasites. Similarly, other helminth infections result in increased antibody levels which do not provide effective immunity, as is the case for *Schistosoma mansoni* (MacDonald et al. 2002), or even seem to correlate with susceptibility, as found for *Taenia solium* (de Aluja et al. 1999). Prime candidates as effector molecules or cells are those that increase in production or number with an infection (Viney 2002), but in most cases the actual effector mechanisms confering resistance remain unsolved. Arguably, protective mechanisms are combinatorial, encompassing non-specific and cellular factors, as well as humoral components. In fish, defence mechanisms against parasites include, in addition to immunoglobulin, complement and other non-specific factors such as lectins, acute phase reactants, lysozyme and antimicrobial peptides, as well as several cell types (Woo 1992, Buchmann & Lindenstrøm 2002).

Antibodies have been detected against some monopisthocotylean monogeneans: for instance against dactylogyrids (Vladimirov 1971) and pseudodactylogyrids (Buchmann 1997, Mazzanti et al. 1999). The differential susceptibility to the nematode *Anguillicola crassus* of Japanese eel *Anguilla japonica*, compared

with the European eel *A. anguilla* has been correlated to antibody titres (Nielsen 1999). Similarly, this work presents evidence of increased antibody titres in partially protected fish. However, considering naïve trout also had detectable antibody levels, further studies are needed to elucidate whether, or to what extent, immunoglobulin alone or in combination with other factors affects the integrity of *Discocotyle sagittata.*

Acknowledgements. We are grateful to Prof. J. L. Molinari (UNAM, Mexico) for advice on the preparation of the parasite extracts. We thank J. Ballard and K. Jervis for access to fish in the Isle of Man. M.R.G. is supported by CONACYT (Mexico) & ORS (UK) postgraduate scholarships.

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Editorial responsibility: Wolfgang Körting, Hannover, Germany

Submitted: April 30, 2002; Accepted: February 2, 2003
Proofs received from author(s): May 28, 2003