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The scaling of dose with host body mass and the determinants of success in experimental cercarial infections

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ABSTRACT

Experimental studies of parasite transmission can help to elucidate life cycles, measure the success of infective stages under different conditions, or test the efficacy of vaccination or other forms of protection against parasitic infection. By combining the results of experiments on a particular parasite taxon, one may also answer questions such as how experimental infection doses are chosen, or what determines infection success. Here, focusing on trematodes, analyses are conducted on data compiled from a total of 145 cercarial infection experiments (62 on non-schistosomes, 83 on schistosomes) obtained from 115 studies. All of these involved experimental exposure of individual hosts to a single known dose of cercariae under controlled laboratory conditions. Across these studies, the cercarial dose used showed a strong positive relationship with the body mass of the target host, independently of the taxonomic identity of that host or of the method of infection used. Although justification for the chosen dose was rarely given, the larger the target host, the more cercariae it was exposed to. Across all experiments, there was also evidence for a weak but significant dose-dependent effect on infection success: the higher the dose used in an experiment, the smaller the proportion of cercariae recovered from the host. This effect was mitigated by either host body mass (for schistosomes) or host taxonomic identity (for non-schistosomes), with infection being lower in fish than in other host types. Experimental procedures also impacted significantly on infection success, namely the infection method used (for schistosomes) and the time between infection and recovery of parasites (for non-schistosomes). Overall, this analysis of published experimental results provides evidence of both biological processes and confounding methodological effects, and it provides strong arguments for greater rationale in the design of experimental infection studies.

1. Introduction

Experimental studies of transmission events have proven extremely useful to our understanding of parasite ecology and epidemiology. They have served to elucidate or confirm life cycles, to measure the performance of infective stages under different conditions, or to assess the suitability of various host species for particular parasite species. Experimental studies have also been extremely useful to test the efficacy of vaccination or other treatments as protection against parasitic infection. Just as these individual studies have provided useful information on their own, an analysis of their pooled results may yield even further insights. For instance, a global look at all studies involving a particular parasite taxon may reveal key determinants of infection success. In addition, this kind of analysis can also serve to evaluate how choices made by researchers when designing a study can impact on its results. For example, the number of infective stages, or the dose, to which hosts are exposed during an experiment is determined by researchers in a manner that sometimes appears arbitrary, and yet the dose of infection can affect estimates of infection success, parasite virulence, or within-host interactions among parasites (Regoes et al., 2003; de Roode et al., 2007; Ben-Ami et al., 2008; Fellous and Koella, 2009).

The present analysis pools data from a large number of published studies of cercarial transmission in trematodes to investigate the impact of decisions made by researchers and also to extract general patterns that provide new information on the transmission biology of these parasites. Trematodes multiply asexually within their first intermediate host, usually a snail (Galaktionov and Dobrovolskij, 2003). The larval stages, or cercariae, thus produced leave the snail to either penetrate and encyst (as metacercariae) within a second intermediate host, or, in the case of blood flukes (family Schistosomatidae), directly penetrate the definitive host. Because of the relative ease with which cercariae can be obtained from infected snails, this stage in the life cycle of trematodes has been extensively studied in the laboratory. Although in nature hosts accumulate cercariae one or a few at a

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time over long periods (trickle infections), in experimental studies a single, one-off exposure to a large number of cercariae is the norm, presumably because it is logistically easier than a series of repeated exposures. The numerous studies using this basic design make it possible to test for general effects of cercarial dose and other experimental parameters on infection success. Apart from the study of Coyne and Smith (1991), which used similar data from experiments on a species of schistosome to estimate worm mortality as a function of infection dose, there has been no attempt to extract patterns from the numerous available studies.

The main question regarding infection experiments involving cercariae concerns the choice of an infection dose. Ideally, it should never produce intensities of infection exceeding those observed in the target hosts in nature, but in reality very little information is provided in most experimental studies to justify this choice. One possible hypothesis is that the chosen cercarial dose increases as a function of the body size of the target host, as a result of decisions made by researchers based on intuition. Larger animal species typically harbour a greater biomass of parasites (Poulin and George-Nascimento, 2007), and larger second intermediate hosts also harbour more metacercariae than smaller ones (Poulin, 2000). Choosing a higher dose for a larger animal therefore seems logical. The shape of the interspecific relationship between cercarial dose and host body size can also be revealing. In practise, it should be described by a power function of the form $D = aM^b$, where D is cercarial dose, M is host body mass, a is a normalisation constant, and b is a scaling exponent. This power function can be converted into a straight line if we plot log-transformed D against log-transformed M, with the scaling exponent b becoming the slope of the linear equation (Harvey, 1982). If the slope b is equal to 1, then the cercarial dose chosen scales in perfect proportion to host body mass. Cercariae penetrate a host through its external surfaces, and thus surface area may be more relevant than body mass when choosing an infection dose. Given that the surface area of animals increase with $M^{0.67}$ (Peters, 1983), if the slope b is closer to 0.67 then researchers tend to choose cercarial doses that match more closely differences in surface area among different hosts than differences in mass. A quantitative examination of this relationship can therefore reveal the subconscious rules followed by people selecting experimental cercarial doses.

Perhaps more importantly, analyses of the combined results of experimental infections can identify the determinants of parasite success. These include both biological processes and methodological influences. On the one hand, infection success is likely to depend on the number of cercariae to which the host is exposed, and on the characteristics of the host itself. Interactions between cercariae during or after host penetration can result in dose-dependent reductions in infection success. These may not be measurable within the range of doses used in a particular study (e.g., Anderson et al., 1978), but can become apparent when a larger dataset is available. Dose-dependence can be mitigated in larger hosts, which offer more space and resources to incoming cercariae, and it may also be influenced by the taxonomic identity of the host, since immune responses against cercariae are probably not uniform across all animals. On the other hand, several aspects of the experimental methods can also impact the success of cercariae. For instance, the duration of the exposure varies from study to study, and one would expect that a greater proportion of cercariae should succeed at infecting the host if given more time, at least up to the duration of the short cercarial lifespan. Similarly, the time between infection and dissection of the host to recover and count the successful parasites also varies across studies; if mortality of parasites post-infection significantly accrues over time, then this factor will also affect estimates of infection success.

The goal of this study was to extract new information from a compilation of experimental data in order to identify general patterns in the infection success of cercariae of both schistosome and non-schistosome trematodes. The specific objectives were (i) to determine whether the choice of infection doses in cercarial experiments is directly related to the body size of the target host, or to other factors such as the host's taxonomic group; (ii) to evaluate the importance of dose-dependence on cercarial transmission, by measuring the impact of the chosen infection dose and the host body mass on cercarial success; and (iii) to determine whether the method of infection, the duration of exposure, and/or the time until dissection affect the estimates of cercarial success. To my knowledge this is the first attempt to analyse data from published experiments on cercarial transmission, and it not only sheds some light on trematode ecology, but it also highlights some effects of experimental methods that should be taken into account in the design of future studies.

2. Materials and methods

2.1. Data collection

A search of the ISI Web of Science® at the end of May 2009 using the search terms 'cercar* and infect* and experiment*' produced a list of 498 studies. All those available through the University of Otago's library system were examined, and data from these publications were included only if they involved experimental exposure of individual hosts to a single known dose of cercariae under controlled laboratory conditions. In all cases, cercariae used represented a genetic mixture, i.e. each host was exposed to a mixture of cercariae issued from multiple snail first intermediate hosts. Studies in which either groups of hosts were exposed together to a known number of cercariae, individual exposure was used but the exact cercarial dose was not given, or individual hosts were exposed to repeated small doses (trickle infection), were excluded. Some studies provided more than one entry in the dataset, by either providing data for different combinations of host and parasite species, or by presenting data on the same host–parasite combination but from distinct experiments. When cercariae of the same species were exposed to hosts of different species in the same experiment, and when one or more host species proved unsuitable for the parasite (because of unusually low infection success), data from those host species were excluded. Many studies measured infection success following the exposure of cercariae to dyes, pesticides, elevated temperatures, etc., or after hosts had been vaccinated or treated in some other way; in these cases, only data from control groups of untreated cercariae and untreated hosts were used. Finally, if experiments were repeated with cercariae of different ages, only data from the most successful cercarial age groups were used.

For each of the remaining experimental results, the following information was recorded: (i) the species name and family of the trematode involved; (ii) the species name of the experimental target host and its taxonomic group, i.e. snail, bivalve, crustacean, fish, amphibian, or mammal; (iii) the average body mass of the hosts used, either given in the original study or obtained from other sources, taking into account any information provided on age or length of individuals used; (iv) the cercarial dose, i.e. number of cercariae per individual host, or, when several doses were used in the same experiment, the intermediate or median dose between the minimum and maximum values used (see also below); (v) the infection success, or the mean number of metacercariae, or adult worms in the case of schistosomes, per individual host recovered at dissection and expressed as a percentage of the initial dose; (vi) the exposure time to cercariae, in hours, generally only given for non-schistosomes; (vii) the time to dissection, measured as the number of days between infection and dissection; and

(viii) for schistosomes only, the method of infection. Mammalian hosts are exposed to schistosome cercariae in two general ways. First, the tail or one leg of the anaesthetised host can be immersed in a container of water to which free-swimming cercariae are added. Second, cercariae may be applied in a few drops of water directly to a shaven patch of skin on the anaesthetised host's body, promoting percutaneous infection, or they may even be injected subcutaneously. The method used in each schistosome study (immersion or percutaneous) was recorded, since the two general methods outlined above should a priori be associated with different infection success; cases in which no detail of the infection method were available were recorded as 'method unknown'. In the case of non-schistosomes, the method of infection was always essentially the same: an individual host, non-anaesthetised, was placed in a small container (i.e. small relative to host size) with cercariae, and left for a given period of time.

Infection success, as defined above, represents not only the success of cercariae at penetrating the host, but also their success at migrating to and establishing in their final site of infection, where they are found at dissection. Thus this measure takes into account not only the proportion of cercariae that fail to enter the host, but also the attrition over time among the initially successful ones.

In addition to the above variables, another measure of dose-dependence was computed for the few studies where several doses were used in the same experiment. Data were recorded on the minimum and maximum cercarial doses used (D_{min} and D_{max}, respectively), and on the mean number of parasites recovered at dissection from these initial doses (R_{min} and R_{max}, respectively). If infection success, or the proportion of parasites that establish, changes as a function of the dose, then the ratio $D_{min}R_{max}/D_{max}R_{min}$ (simply called dose-dependence below) should differ from 1. A ratio smaller than 1 would indicate that infection success is smaller for higher doses, whereas a ratio greater than 1 would indicate that infection success increases at higher doses.

Schistosomes and non-schistosome trematodes differ in many ways. Cercariae of schistosomes directly penetrate the definitive host (among the species included here, this was always a mammal), in which they develop into adult worms within the circulatory system. Cercariae of the other trematode families considered here penetrate an intermediate host (either an invertebrate or an ectothermic vertebrate), in which they encyst as metacercariae. In addition, there are methodological differences between experiments on cercarial infection involving schistosomes and non-schistosomes that render comparisons between them difficult. For all these reasons, in the analyses that follow, schistosomes and non-schistosomes are treated separately.

2.2. Statistical analyses

Three continuous variables, host body mass, cercarial dose and time to dissection, required logarithmic transformation to meet the assumptions of normality. Exposure times to cercariae (non-schistosomes only) showed a pronounced bimodal distribution: 24% of exposure times were 1 h or less, whereas 50% were exactly 24 h, with all other values falling in between but generally close to either of these extremes. Therefore, this variable was converted into a binary variable (exposure time either <9 h or 17–24 h).

Generalised linear models (GLM) were used to analyse what factors determine the choice of the cercarial dose used in an experiment, and what factors influenced both infection success and dose-dependence; all were carried out with the statistical software JMP 7.0. For cercarial dose and dose-dependence, the models had errors based on a normal distribution, whereas for infection success the models had a Poisson error structure and log link function. After starting with a full model (see below), significance levels were based on the deviance explained by each factor, based on χ^2 statistics, following backward stepwise elimination of non-significant ($P > 0.05$) terms. Only the final models are presented in the results. All likely two-way interactions were initially included, but none were retained as significant.

In analyses of the choice of cercarial dose, two explanatory variables were included in the initial GLM for non-schistosomes: host body mass and host taxonomic group (snail, bivalve, crustacean, fish or amphibian). For schistosomes, since all hosts used were mammals, the two explanatory variables used were host body mass and method of infection (immersion, percutaneous or unknown). Subsequently, to assess the scaling relationship between host body mass and the chosen cercarial dose in an experiment, the slope of the linear regression (ordinary least squares) between these two variables, both log-transformed, was also computed.

In analyses of the factors influencing either infection success or dose-dependence, five explanatory variables were included in the initial GLM for non-schistosomes: host body mass, host taxonomic group, cercarial dose, exposure time to cercariae and time to dissection. For schistosomes, the four explanatory variables used were host body mass, cercarial dose, method of infection and time to dissection.

3. Results

Data from a total of 145 cercarial infection experiments were obtained from 115 studies; the full dataset, including the list of original studies, is available in Supplementary Table S1. These consist of 62 experiments on non-schistosomes, representing 13 families, and 83 experiments on schistosomes, all of which involved members of the genus *Schistosoma* (Table 1). Among non-schistosomes, the family Echinostomatidae was by far the best represented in the dataset (38 out of 62 experiments; Table 1). These were treated separately in preliminary analyses to determine whether they biased the results; since the outcomes of these preliminary analyses were highly consistent with those of analyses including all non-schistosomes (see below), only the latter are reported here.

Values for infection success were roughly comparable between non-schistosomes (mean ± standard error, 41.9 ± 2.9%) and schistosomes (34.2 ± 2.2%), though a little higher in the former than in the latter (Fig. 1). However, in addition to the biological and methodological differences highlighted in Section 2.1, non-schistosomes and schistosomes differed in three other respects in the experiments considered here. First, lower cercarial doses were used in experi-

Table 1
Summary of the taxonomic composition of the dataset on experimental cercarial infections.

Trematode family	Number of experiments	Number of trematode species (genera)	Number of host species	Host taxa[a]	Number of studies
Cryptogonimidae	1	1 (1)	1	F	1
Diplostomatidae	5	3 (3)	3	A, F	5
Echinostomatidae	38	14 (6)	19	B, S, A	23
Hemiuridae	1	1 (1)	1	C	1
Heterophyidae	2	2 (1)	2	F	2
Microphallidae	3	1 (1)	1	C	3
Opecoelidae	1	1 (1)	1	C	1
Paragonimidae	1	1 (1)	1	C	1
Plagiorchiidae	1	1 (1)	1	A	1
Psilostomidae	2	1 (1)	2	A	2
Renicolidae	2	2 (2)	2	B	2
Sanguinicolidae	3	1 (1)	1	F	3
Transversotrematidae	2	1 (1)	2	F	2
Schistosomatidae	83	6 (1)	16	M	70

[a] Host taxonomic groups: C, crustaceans, B, bivalves, S, snails, A, amphibians, F, fish, M, mammals.

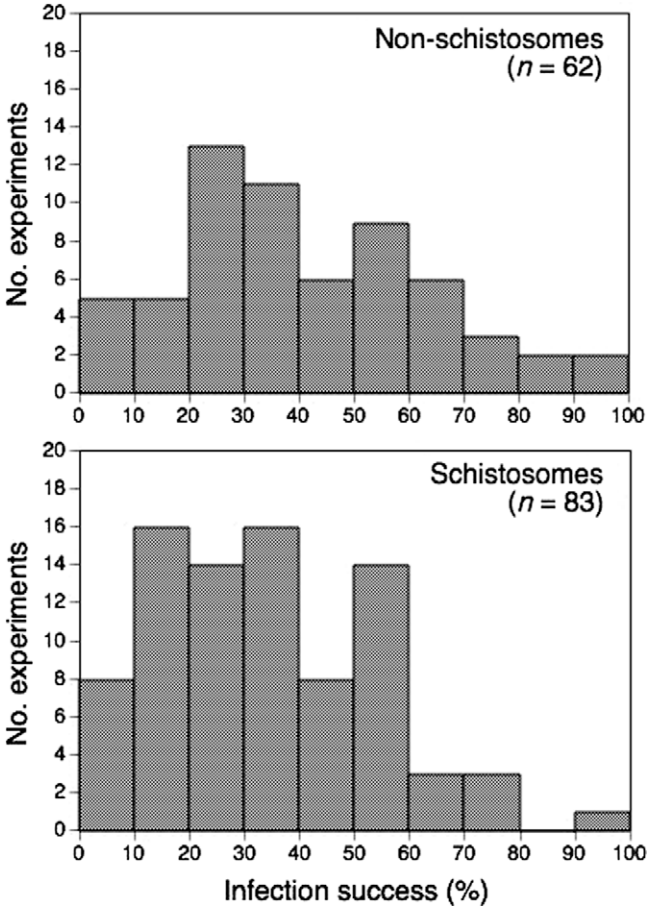

Fig. 1. Frequency distribution of infection success values in experiments involving cercariae of non-schistosomes and schistosomes. Infection success is the mean number of worms per individual host recovered at dissection and expressed as a percentage of the initial dose.

ments with non-schistosomes (range 4–1,500; median = 25) than with schistosomes (range 20–10,000; median = 150). Second, non-schistosomes were used to infect much smaller hosts (range from <0.1 to 73.5 g; median = 0.27 g) than those exposed to schistosomes (range from 3 to >100,000 g; median = 20 g); the former parasites were generally used to infect small crustaceans or snails, or young tadpoles, whereas the latter were used to infect mammals. Third, times from infection to host dissection were also shorter for non-schistosomes (range 0.75–120 days; median = 5 days) than for schistosomes (range 14–240 days; median = 56 days). These differences provide further justification for treating these two groups of trematodes separately.

3.1. Choice of cercarial dose

For non-schistosomes, host taxonomic group was not included in the final model, and only host body mass influenced the cercarial dose chosen for an experiment ($\chi^2 = 21.99$, df = 1, $P < 0.0001$). Similarly, for schistosomes, method of infection was not retained in the final model, and only host body mass influenced the cercarial dose chosen ($\chi^2 = 88.61$, df = 1, $P < 0.0001$). For both groups of trematodes, higher cercarial doses were chosen for larger-bodied host species (Fig. 2).

When pooling all trematodes, the slope of the strong positive relationship ($r^2 = 0.621$; Fig. 2) between host body mass and cercarial dose chosen, based on ordinary least squares regression with log-transformed data, was 0.297 (95% confidence intervals

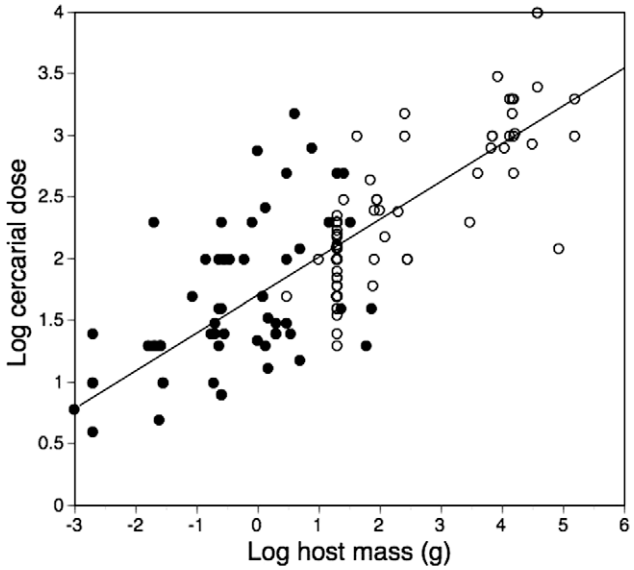

Fig. 2. Relationship between host body mass and the cercarial dose chosen for an experimental infection, for both non-schistosome (filled symbols; $n = 62$) and schistosome (open symbols; $n = 83$) trematodes. The line represents the best-fit line from a linear regression.

(CI) = 0.259–0.335). Schistosomes and non-schistosomes were pooled because the slopes of the separate relationships for each group (non-schistosomes: 0.269, 95% CI = 0.164–0.375; schistosomes: 0.371, 95% CI = 0.312–0.430) were not different, i.e. their 95% CIs overlap substantially. The relationship across all trematodes spanned eight orders of magnitude in host body mass (Fig. 2). The observed slope value differed from the slope expected from direct proportionality with host body mass (slope = 1) or from proportionality with host body surface area (slope = 0.67) (see Section 1). This suggests simply a general correlation with host body mass.

3.2. Infection success and dose-dependence

For non-schistosome trematodes, host body mass and exposure time were not included in the final model explaining infection success. However, initial cercarial dose, time to dissection and host taxonomic group all significantly affected infection success (Table 2). Overall, infection success tended to be lower with increasing cercarial doses (Fig. 3), and to increase with longer times until dissection of experimentally exposed hosts. Also, infection success was usually low in fish hosts and reached its highest average values in molluscan and amphibian hosts (Fig. 4). Estimates of

Table 2
Results of generalised linear models of the factors influencing infection success and dose-dependence in experimental cercarial infections. Only the final models are shown, following backward elimination of non-significant factors. The χ^2 tests assess the significance of the deviance explained by each factor.

Response variable	Factor	df	χ^2	P
Non-schistosomes				
Infection success	Cercarial dose	1	80.32	<0.0001
	Time to dissection	1	10.40	0.0013
	Host taxonomic group	4	29.34	<0.0001
Dose-dependence	Cercarial dose	1	3.95	0.0469
Schistosomes				
Infection success	Cercarial dose	1	21.25	<0.0001
	Host body mass	1	4.03	0.0446
	Method of infection	2	19.72	<0.0001

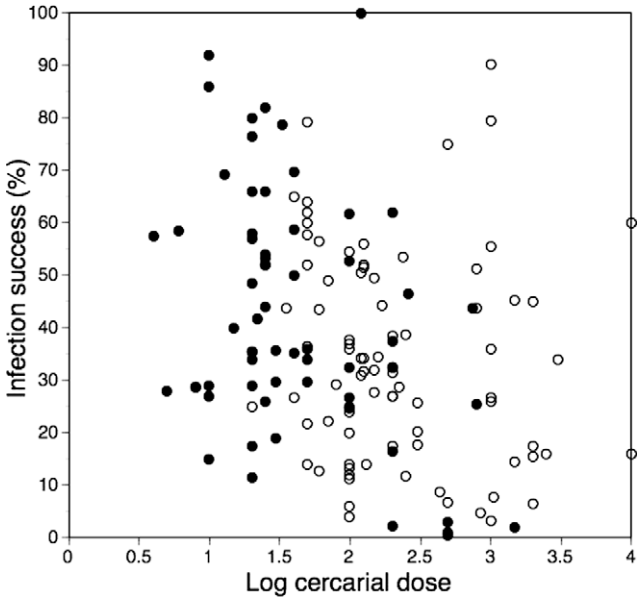

Fig. 3. Relationship between the cercarial dose chosen for an experiment and infection success, i.e. the mean percentage of worms recovered per individual host. Data are shown separately for both non-schistosome (filled symbols; $n = 62$) and schistosome (open symbols; $n = 83$) trematodes.

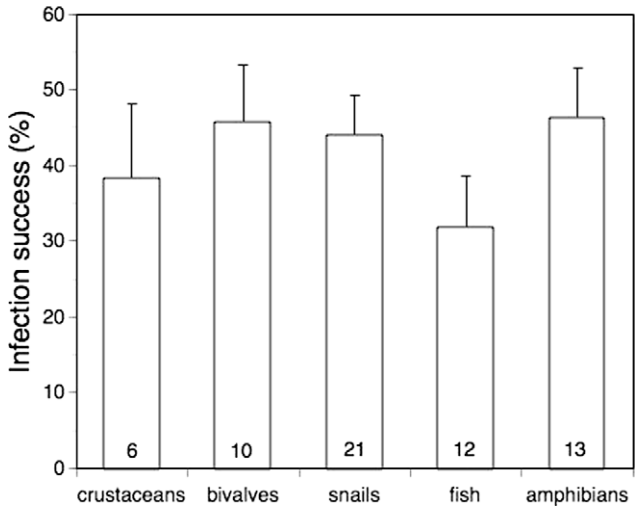

Fig. 4. Mean (±standard error) infection success of non-schistosome trematode cercariae in different taxa of second intermediate hosts under experimental conditions. Numbers on each bar indicate the number of experiments with each host taxon.

dose-dependence were only available from 10 experiments, which limited the power of the analysis of the factors influencing dose-dependence in non-schistosomes. Nevertheless, cercarial dose was the only one retained in the final model (Table 2). Dose-dependence was estimated from doses both smaller and larger than the intermediate dose used here as the measure of 'cercarial dose' (see Section 2.1). Among the experiments for which data were available, dose-dependence tended to be weaker for higher values of the intermediate cercarial dose (Fig. 5), indicating that experiments using higher average doses result in proportionately lower infection success.

For schistosomes, only time until host dissection was not retained in the final model explaining infection success. Initial cercarial dose, host body mass, and method of infection all significantly affected infection success (Table 2). As for other trematodes, infection success tended to be lower with increasing cercarial doses (Fig. 3). Infection success also tended to increase slightly with increasing host body mass, and was generally higher when infection was performed by the percutaneous method than by immersion of host body parts (leg or tail) in water containing cercariae. In the analysis of dose-dependence and its determinants, none of the factors considered (host body mass, cercarial dose, method of infection and time to dissection) had a significant effect on estimated dose-dependence, although it must be pointed that data on dose-dependence were only available from eight experiments.

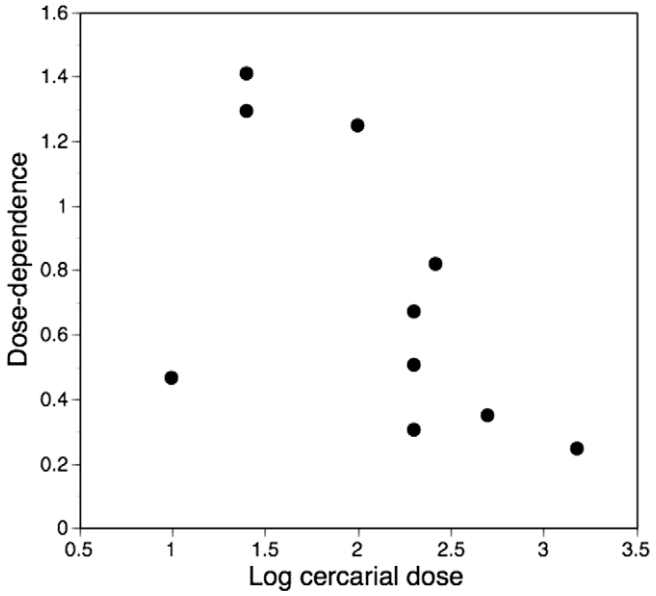

Fig. 5. Relationship between the cercarial dose chosen for an experiment and dose-dependence for non-schistosome trematodes. Here, cercarial dose is best seen as the intermediate or median value between the highest and lowest doses used in a particular experiment. The smaller the value of the dose-dependence index, the greater the reduction in infection success as a function of increasing cercarial dosage (see Section 2.1 for details on its computation).

4. Discussion

Experimental infection under controlled conditions is a powerful tool in parasitology, allowing researchers to re-create and closely examine key transmission processes in the laboratory. In the case of trematode cercarial transmission, experimental infections have provided vital information on previously unknown life cycles (Simoes et al., 2008), the determinants of transmission success (Koprivnikar et al., 2006; Thieltges and Rick, 2006; Griggs and Belden, 2008), parasite impacts on host fitness (Fredensborg et al., 2004) and the efficacy of vaccines against schistosomes (Attallah et al., 2004; Ganley-Leal et al., 2005). However, the results of cercarial infections depend on decisions made by researchers during the design of the experiment. These include how many cercariae to use, how long to expose the host to cercariae, and how long to wait until recovering the successful parasites. The present analysis has focused both on the choices made by researchers and the general determinants of infection success, and extracted three general patterns from a compilation of available studies: (i) larger experimental hosts are invariably given larger cercarial doses; (ii) infection success by both schistosomes and non-schistosomes is generally negatively related to the initial dose, although this dose-dependent effect can be mitigated by either the size or taxonomic identity of the target host; and (iii) infection success is also highly dependent on methodological procedures.

Intuitively, one would expect a larger host to be able to withstand a higher cercarial dose; however, if experiments are to yield meaningful data, the choice of dose should be based on what the animals actually experience in nature, and not on what they appear capable of enduring. Cercarial density, i.e. dose divided by the water volume in which exposure took place, might actually be a better measure than dose itself. However, accurate data on water volumes were only available for some studies, and since the experimental containers used for non-schistosomes always constrained host movement, dose remains a valid measure of cercarial exposure. Across all experiments available for analysis, fewer than 10% provided a justification for the cercarial dose chosen. Across all studies, the body mass of the target host explained about 62% of the variability in cercarial dose, making it a strong predictor of what dose researchers are likely to choose. For schistosomes, the method used to expose hosts to infection had no significant effect on the choice of dose. For non-schistosomes, the taxonomic identity of the target host, i.e. whether it was an amphipod, a clam or a fish, did not have a separate influence on the dose chosen beyond the fact that these animals have different body masses. The scaling coefficient between cercarial dose and host body mass (0.297) is much lower than what would be expected if the dose was chosen to be exactly proportional to either host mass or surface area. Of course, neither host mass nor host surface area is expected to fully explain patterns of trematode infections in nature. For instance, in some hosts such as crabs, the area suitable for cercarial penetration (i.e. the gills) is much less than total body surface area, and the latter may thus not be a good predictor of infection levels (Saville and Irwin, 2005).

Nevertheless, proportionality of experimental doses with either host mass or surface area would hint at some rationale for the choice of cercarial doses. The evidence suggests instead that researchers just choose higher doses for larger animals without following any explicit or rigorous quantitative rule. This may also apply to other aspects of the design of experiments involving cercarial infection; indeed, for schistosomes, host body mass correlated significantly with the time between infection and dissection (both log-transformed: $r = 0.368$, $n = 81$, $P = 0.0007$). Subjective decisions based on the size of the model animal thus appear to influence experimental design in many ways. Other factors, not investigated here, could also easily influence experimental decisions; for example, perhaps the use of a trematode species in which few, very large cercariae are produced per unit time would lead to the selection of a smaller dose than if a trematode producing numerous, very small cercariae were used instead, for no other reason that one of convenience. Similarly, maybe the lifespan of cercariae limits the exposure time that can be chosen.

Yet all these decisions matter. The dose chosen is particularly crucial, since the other major finding of this study is that across all experiments, there is evidence that infection success is negatively dose-dependent: proportionally fewer cercariae are successful at infecting the host as the dose is increased. Evidence for this comes from the significant effect of cercarial dose in the GLMs on infection success in both schistosomes and non-schistosomes, as well as the separate analysis of dose-dependence in non-schistosomes for the few studies that used a range of infection doses. This effect could result from interactions among cercariae during the infection process as they jostle for position on parts of the host body suitable for penetration, or if they trigger a host grooming response beyond a certain dose. Alternatively, the effect could result from post-infection interactions, if suitable sites within the host body become saturated by a large number of cercariae arriving more-or-less simultaneously. Although statistically significant, this dose-dependent effect was not particularly strong (see scatter in Fig. 3), and it is sometimes not seen within individual studies (e.g., Anderson et al., 1978); therefore, clearly other factors can influence infection success.

One of these factors is the nature of the target host. In non-schistosomes, the taxonomic identity of the host had an effect on infection success, with cercariae generally achieving lower success in fish than in other types of target hosts. Fish can mount immune responses against cercariae (e.g., Lysne et al., 1997), but the same should be true of other types of target hosts, such as crustaceans (see Bryan-Walker et al., 2007). Perhaps the efficacy of grooming movements or other forms of behavioural defences differs between fish and other types of target hosts. Further research will be needed to confirm and explain the uneven performance of non-schistosome cercariae on different types of hosts. For schistosomes, where the target host was always a mammal in the available studies, it was host body mass that seemed to matter for infection success. Generally, for a given cercarial dose, schistosome success tended to increase slightly with increasing host body mass. Given that schistosomes live in restricted sites within the host, i.e. specific parts of the circulatory system, larger hosts should provide more living area for the worms, which should dampen any post-infection competitive interactions among individuals and lead to the recovery of a greater proportion of the initial dose. This was a relatively weak effect, however, probably only detectable because host body masses in schistosome experiments spanned five orders of magnitude.

In addition to the biological factors discussed above, methodological issues can also impact upon estimated infection success. Even under laboratory conditions thought to be ideal for transmission (i.e. chances of contacting the host are maximised, no interference from other organisms, etc.), most studies resulted in fewer than 50% of cercariae successfully infecting their target host (see Fig. 1). In non-schistosomes, the time of exposure, i.e. the time allocated for the cercariae to contact and penetrate the host, did not affect infection success. This is somewhat surprising, and it must mean that although some exposure times were very short, in combination with the use of small experimental containers, they were nevertheless long enough to allow maximum contact with the host. In contrast, infection success was higher when a longer time was allowed until dissection of infected hosts. This seems counter-intuitive, since mortality of cercariae post-infection should generate the opposite trend. However, cercariae require time to migrate from the site of penetration to the final site of encystment as metacercariae. Many researchers, at the time of dissection, focus on the host tissues where metacercariae are expected, and thus early dissection may miss worms still in transit. The relationship between the proportion of worms recovered and time to dissection has been highlighted in individual studies (e.g., Lyholt and Buchmann, 1996), and choosing a suitable time until dissection needs to be considered carefully in the design of cercarial experiments.

In schistosomes, in contrast, time until the host is dissected and perfused for worm recovery was not significantly associated with estimated infection success, most likely because the times were generally much longer, certainly long enough to allow the worms to establish within blood vessels. However, the method used for infection affected infection success: proportionally more cercariae were recovered when infection was performed by the percutaneous method than by immersion of host body parts (leg or tail) in water containing cercariae. Percutaneous methods (such as the ring method; Smithers and Terry, 1965) that place cercariae directly in contact with host skin should maximise penetration relative to methods that allow cercariae to swim freely without guaranteeing contact. Previous comparisons of the efficacy of these methods have found no major differences in infection success between them (Christensen et al., 1977), but the present meta-analysis suggests that there is a difference. Clearly, the choice of method for schistosome infections must be made with care, especially if one wants to compare results of one study with those of

others to assess the efficacy of vaccines or other potential control methods.

In summary, the present meta-analysis of published studies of experimental trematode infection has highlighted some clear general patterns. On the one hand, cercarial infection success shows weak but pervasive dose-dependence, although the negative effect of higher doses on relative infection success can be modulated by either the taxonomic group of the target host (for non-schistosomes) or its body size (for schistosomes). On the other hand, the choice of experimental methods in published studies is both poorly justified and strongly influential on the estimated success of cercarial infection. These findings do not cast doubts over the usefulness of experimental infections, but they do emphasize the need for careful consideration of how methodological artefacts can confound the measurement of biological processes related to parasite transmission.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ijpara.2009.09.001.

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