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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