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Effect of fish size on transmission of fish-borne trematodes (*Heterophyidae*) to common carps (*Cyprinus carpio*) and implications for intervention

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ABSTRACT

Fish-borne trematodes are reported to affect the health of more than 40 million people worldwide. Few experimental studies are available on fish size dependent gain (attack rates of cercariae) or loss (mortality of metacercariae) of fish-borne trematodes. Aim was to quantify the relation between fish size and attack rates of fish-borne trematodes in common carps (*Cyprinus carpio*). Effect of fish size and cercariae dose were tested in a 3×4 factorial design with 5 fish per combination of treatments (n=60). Individually kept small (1 g), medium (25 g) and large (45 g) carps were exposed to 0, 10, 50 or 250 parapleurolophocercous-cercariae (*Heterophyidae*) for 48 h. Fish were digested 21 days post exposure to count metacercariae. Percentages of fish containing metacercariae, and attack rates of cercariae to fish were higher (63%, 0.08 fish infected per cercariae) for small common carps than for medium (20%, 0.004 fish infected per cercariae) and large common carps (5%, 0.0007 fish infected per cercariae), but never zero. It was concluded that exposure of small fish is an important risk factor for transmission of fish-borne trematodes. The results suggest that control measurements aimed at reducing transmission to small fish may considerably reduce the absolute amount of fish-borne trematodes.

1. Introduction

Fish-borne trematodes of the family *Heterophyidae* belong to the phylum *Platyhelminthes*, or flatworms. Life cycles of fish-borne trematodes involve three types of hosts. Primary aquatic snail hosts, secondary fish hosts and final hosts, like human, pigs and chicken (Sithithaworn et al., 2008). The health of more than 40 million people worldwide is affected by these trematodes, especially in South-East Asia and the Western Pacific (Abdussalam et al., 1995; WHO, 1995, 2002). About 70 species are known to be zoonotic (WHO, 2002), of which the most described species, *Opisthorchis viverrini*, *Clonorchis sinensis*, and *Opisthorchis felineus*, have been estimated to infect 17 million people (Sithithaworn et al., 2008). Raw or undercooked fish dishes are the major sources of human infection (Sithithaworn et al., 2008).

More than hundred fish species are susceptible to fish-borne trematodes (WHO, 1995). Between fish species, prevalence and parasite burden of *C. sinensis* are in general higher in small species compared to larger species (Komiya, 1966; Rim, 2005). Within species, young fish are assumed to be more susceptible to infection with fish-borne trematodes because of their relative thin skin and lack of previous exposure (reviewed by Lun et al., 2005). However, observational studies that take fish size into account were not consistent in their results. In larger fish, more trematodes were found by Kimura and Uga (2005). No relationship was found by Wang et al. (2002). Thien et al. (2007) observed several fish species and found both higher prevalence and higher burden in small fish compared to larger fish as well as no effects. They suggested that species-specific factors might be important for fish-borne zoonotic trematodes transmission. Thuy et al. (2010) observed higher prevalences with increasing age; fish weights were unknown. Phan et al. (2010) found no infections in fish fry from hatcheries, and increasing prevalences from 14% infected one-week-old juveniles up to 58% in overwintered juveniles.

Observational studies, however, lack quantitative information about previous exposure of fish to cercariae. Prevalence and parasite burden of fish-borne trematodes in fish obtained at a specific point in time results from two processes: previous gain and loss of metacercariae. Fish gain metacercariae by a certain attack rate of cercariae to fish and lose metacercariae due to mortality of metacercariae in fish. Cercariae dose in water might be assumed to vary under normal pond conditions, since presence and amount of snail hosts vary among sites (Dung et al., 2010). Cercariae shedding by snails is known to be unpredictable and irregular (Lo and Lee, 1996; Schreiber and Schubert, 1949). Furthermore, the effect of light, temperature, water flow and other environmental factors on cercariae shedding is not clear (Lo and Lee, 1996; Phongsasakulchoti et al., 2005). This implies that for the relationship between metacercariae and fish size observed during observational studies attack rates of cercariae to fish cannot be estimated. Therefore, attack rates might be determined by

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experimental exposure only. In one experimental study, attack rates of cercariae of the eyefluke *Diplostomum spathaceum* were higher in rainbow trout (*Oncorhynchus mykiss*) of 5–7 cm than in rainbow trout of 12–15 cm when water volumes were proportional to fish surface areas (Hoglund, 1995). However, no other experimental studies are available on fish size dependent attack rates of cercariae or mortality of *Heterophyidae* metacercariae in fish.

Therefore, the aim of our experimental study was to quantify the effect of fish size on the percentage of fish with metacercariae and attack rates of fish-borne trematodes (*Heterophyidae*) in common carps after experimental exposure to different doses of cercariae.

2. Material and methods

2.1. Experimental design, fish and parasites

Effect of fish size and cercariae dose was tested in a 3×4 factorial design with five fish per treatment (n = 60). Common carps (*Cyprinus carpio*) were used as host fish because they are known as intermediate hosts for fish-borne trematodes and are used in traditional dishes (Phan et al., 2011; WHO, 1995). Small, medium and large common carps were purchased from one local fish farmer 6 days prior to start of the experiment. Age and history of fish was unknown. Until exposure, small, medium, and large fish were kept in two tanks containing 48 l well-water. Mean fish weight (SD) was 1.1 (0.4) g for small, 24.3 (4.8) g for medium, and 45.7 (8.8) g for large fish. Fish were fed commercial fish food at 5% body weight. No feed was provided from 24 h before to 12 h after exposure to cercariae.

Melanoides tuberculata snails were obtained one month prior to the experiment from households in Ngia Lac, Nam Dinh Province, Vietnam, where a high prevalence of *Heterophyidae* was observed (Anh et al., 2010; Dung et al., 2007, 2010; Phan et al., 2010). Snails were individually kept in glass cups filled with about 5 ml well-water for 2 h to shed cercariae. Most *Heterophyidae* cercariae are parapleurolophocercous-cercariae (Schell, 1985), therefore only snails shedding parapleurolophocercous-cercariae were used. Cercariae were identified and counted under a stereomicroscope as they were drawn into a pipet. Pipets were emptied in petri-dishes and number of cercariae was recounted. Petri-dishes containing 10, 50 or 250 cercariae were emptied in tanks with a bottom surface of 11×11 cm containing 500 ml well-water.

Fish were kept and exposed individually to 0, 10, 50 or 250 cercariae for 48 h, all at the same date. During exposure, an air-stone was provided to each tank. After exposure, fish were dipped in well-water for 1 minute to wash remaining cercariae off the fish. During 21 days after exposure, fish were individually kept in net cages in tanks containing 3000 l of cercariae free water. Water temperature was recorded daily, and was on average 21 °C, varying between 18 and 22. Every tank contained 12 net cages containing one fish each. Dead fish were collected two times daily, and stored at 5 °C until digestion.

2.2. Parasite recovery

Metacercariae were recovered from fish within 36 h post mortem or at the end of the experiment 21 days after exposure to cercariae. Before digestion, weight and total length of the fish were recorded. Digestion protocol was an adjusted version of the digestion method described in annex 6 of WHO (1995). Fish were individually grinded in artificial stomach acid solution consisting of 0.06 M HCl and 1% pepsin in distilled water, with a ratio of 100 g of fish per liter artificial stomach acid. The mixture was incubated for 2–3 h at 37 °C, after which the digested material was filtered through a 1×1-mm mesh brass sieve and washed several times with 0.85% saline solution until the supernatant became clear. The sediment was searched twice by stereomicroscope. Recovered metacercariae were collected, counted and identified by microscope at 500x magnification, based on morphological characteristics described by Scholz et al. (1990, 1991) and according to the FIBOZOPA laboratory manual (2006, unpublished).

2.3. Statistical analysis

Survival analysis was used to study the effect of fish size and cercariae dose on survival time of fish. Survivor functions were estimated using the Kaplan-Meier method, and compared with the log rank test (PROC LIFETEST, SAS Inst. Inc., 2004).

Infection (yes/no) was analyzed by (exact) logistic regression (PROC LOGISTIC, SAS Inst. Inc., 2004) in which effect of fish size (small, medium, large), cercariae dose (0, 10, 50, 250), and their interaction were tested. A fish was considered infected when at least one metacercariae was recovered. Results were expressed as prevalences and Odds Ratios. An Odds Ratio (OR) is a measure of the association between exposure and infection; an OR>1 indicates that a variable increases the risk of infection; an OR<1 that it decreases the risk (Noordhuizen et al., 2001).

The number of recovered metacercariae was analyzed with generalized linear models with a Poisson distribution and log link function (PROC GLIMMIX, SAS Inst. Inc., 2004). Effect of fish size, infection dose, and their interaction were included in the model as explanatory variables.

For undeveloped metacercariae, we calculated the attack rate in two ways. First, using the number of cases (fish with metacercariae), second using metacercariae burden.

In the first method, data were statistically analyzed using generalized linear models to estimate the attack rate λ. A binomial distribution with a complementary-log–log link function was used, with offset:

log(cercariae exposure dose + 1) (PROC GENMOD, SAS Inst. Inc., 2004). Expected number of fish with metacercariae (E(C)) is then equal to:

$$E(C) = \left(1 - e^{(-\lambda \cdot (\text{cercariae exposure dose} + 1))}\right) \cdot S,$$

or

$$E(C/S) = \left(1 - e^{(-\lambda \cdot (\text{cercariae exposure dose} + 1))}\right),$$

in which S is the number of susceptible fish at start of the experiment. Taking the log of this model results in:

$$\log E(C/S) = \log(\lambda) + \log(\text{cercariae exposure dose} + 1).$$

Fish size (small, medium, large) was included in the model as fixed effect. The estimated parameter is log(λ); exponentiation gives λ and represents the number of infected fish caused by one cercariae as result of exposure. Controls (infection dose = 0) were excluded because transmission is not possible.

Second, attack rate based on metacercariae burden was defined as the number of new metacercariae recovered per cercariae of the exposure dose. Data were analyzed using nonparametric models because metacercariae burden was not normally distributed. Wilcoxon scores were used to rank the observations for fish size (small, medium, large) (PROC NPAR1WAY, SAS Inst. Inc., 2004).

3. Results

3.1. Survival

In total, 97% of fish survived 48 h of exposure to cercariae. At 21 days post exposure, survival was 38% with resp. 0, 50, and 63%