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Distribution of trematodes in snails in ponds at integrated small-scale aquaculture farms

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ABSTRACT

In integrated small-scale aquaculture farming, animal and human excreta maybe used as fish feed and pond fertilizer, thereby enhancing transmission of fish-borne zoonotic trematodes (FZTs) from final hosts, like humans, pigs and chickens, to snails. Areas within a pond could vary in trematode egg-load due to the immediate bordering land, and this might provide implications for control of these trematodes or sampling in field studies measuring FZT prevalence in snails. We therefore estimated the effect of bordering land use on prevalence and FZT burden in snails in different areas within small-scale aquaculture ponds. Nine sampling areas within a pond were assigned in six ponds. For each sampling area, about 120 *Melanoides tuberculata* snails were collected. Based on land use bordering a sampling area, these were categorized in 5 risk-categories: low-risk (road, rice planted in pond, agriculture, or middle of pond), human access point to pond, livestock sty (pigs or poultry), both human access point and livestock sty, and water connection to canal. In total, 5392 snails were collected. Percentages of snails with parapleurolophocercous cercariae varied between 6% in areas categorized as low-risk and areas with livestock sty only to 15% in areas with both human access point and livestock sty; only this 15% was significantly different from the prevalence in the low-risk category. Percentages of snails with xiphidio cercariae did not differ between risk-categories and varied between 5% and 10%. Mean snail size was 15.2 mm, and was significantly associated with both the probability of infection as well as parasite burden. Very small differences in parasite burden were found at different land use areas; the maximum difference was about 11 cercariae. This study demonstrated only small differences between areas surrounding a pond on risk of snails to be infected with fish-borne trematodes within different pond areas. In field studies on FZTs in *M. tuberculata* snails in ponds, sampling from ponds can therefore be done without considering areas within ponds.

1. Introduction

Fish-borne zoonotic trematodes (FZTs) affect more than 40 million people worldwide (WHO, 2004). Liver flukes (*Opisthorchiidae*) are known best because of their clinical importance (WHO, 2011). Intestinal flukes (e.g. *Heterophyidae*) causes severe pathology in humans (Toledo et al., 2006), but impair food safety and quality, which has consequences for public health (Yu and Mott, 1994; Chai et al., 2005). The life-cycle of FZTs involves three types of hosts, primary intermediate snail host, secondary intermediate fish host, and final host like humans, pigs, cats or fish eating birds (Komiya, 1966; Sithithaworn et al., 2008). In this paper we focus on the role of snails. Asexual reproduction within the snail results in the release of cercariae over a period of months to years (Esch et al., 2002) and these cercariae might cause infection in fish which in turn are the reason for the food safety concern associated with FZTs.

In integrated small-scale aquaculture farming systems, fish ponds are combined with other agricultural activities within the boundaries of one farm (Prein, 2002). Besides much strength, a weakness of small-scale aquaculture farming systems is potential human health risk because of the use of animal and human excreta as pond fertilizers (Phan et al., 2011; Sapkota et al., 2008). These excreta may contain (antimicrobial-resistant) pathogenic bacteria (Petersen and Dalsgaard, 2003), viruses (Sapkota et al., 2008), and macro-parasites (Chai et al., 2005).

On small-scale aquaculture farms in Northern Vietnam (Dung et al., 2010), identical species of fish-borne trematodes were found in snails (Dung et al., 2010), fish (Phan et al., 2010), humans (Dung et al., 2007) and other reservoir hosts (Anh et al., 2010; Lan Anh et al., 2009), suggesting the life-cycle of specific FZTs is maintained on these farms.

Spatial distribution of final hosts have been reported to cause patchiness in trematode infections in snail populations, especially

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when individual snail movement is low and snails get infected by ingesting trematode eggs (Esch and Fernandez, 1994; Hechinger and Lafferty, 2005; Krist et al., 2000; Jokela and Lively, 1995). Fish ponds in Northern Vietnam are typically surrounded by agriculture (crops and/or fruit trees), livestock sty for pigs or poultry with one or more outlets to the fish pond, farmers house with access point to the pond, duck sty with enclosure for ducks in the pond, water connection to a canal, road and rice planted in the pond (Dung et al., 2010; FAO, 2005). These different types of land use bordering a pond could lead to variation in contamination of different pond sections with trematode eggs and with that cause patchiness in FZT infection in snail populations within a pond. Dung et al. (2010) showed variation in prevalence of FZTs in snails among large canals, small canals, rice fields and small-scale aquaculture ponds, but so far information on within pond variation of fish-borne trematodes in snails is lacking. If certain types of land use would be risk factors, then there might be implications for sampling methodology in field studies involving snails and this might give insight in how to design control measures. Therefore, the objective of this study was to estimate the effect of bordering areas on prevalence and parasite burden of trematodes in snails within ponds on integrated small-scale aquaculture farms.

2. Materials and methods

Six ponds with high *Melanoides tuberculata* numbers (>250 snails scooped per person per hour, maximum sample time per pond 4 h) were sampled in September 2009. The ponds were located in Nghia Lac and NghiaPhu communes, Nam Dinh province, Vietnam. Both communes have a history of endemic FZTs and are known for raw-fish-eating behavior of humans and small-scale aquaculture farming (Dung et al., 2010; Phan et al., 2011). The six fish ponds ranged in size from 290 m^2 to 8190 m^2. Rice was only cultivated in pond 4 in about 25% of pond area. One pond was inhabited by ±400 ducks in an enclosure of ±2330 m^2, no snails were found within the enclosure. Further description of ponds is shown in Table 1.

Sample sizes were calculated using WIN EPISCOPE 2.0 (Thrusfield et al., 2001) using 95% confidence (two tailed) and 80% power to detect a difference between 3 and 15% prevalence (Dung et al., 2010) between groups. This resulted in a sample size of 87 snails per group. As 20% of snails would not be alive (Dudgeon, 1986), at least 110 snails per group should be sampled. Based on this we have decided to sample 120 *M. tuberculata* snails per sampling area. Each pond was "divided" in 9 sampling areas, being the 4 corners, 4 sides between corners and the middle of the pond in rectangular ponds. For each of these areas, 1 or 2 types of land use bordering a sampling area were recorded (Table 1). Road, dike, rice cultured in pond, none (middle of the pond), or agriculture (crops and fruit trees) were considered as low-risk land use for infection of snails. Presence of livestock sty (pigs and/or poultry), human access point to pond or water connection to canal were considered as higher risk. Based on type of land use bordering a sampling area, they were categorized in 5 risk-categories: low-risk, human access point to pond, livestock sty, both human access point and livestock sty, and water connection to canal. In case two different types of land use (e.g. livestock sty and agriculture) bordered a sampling area, it was assigned to the risk-category with potentially the highest risk (in the example: livestock sty). It was assumed that both human access point and livestock sty was at highest risk, and was therefore categorized as a separate risk-category.

From each sampling area within a pond, bottom sediment containing snails was filtered with a sieve. Processing of snail samples from 1 pond began in a laboratory about 1 h after sampling and was completed before continuing with the next pond. Snails were individually transferred to approximately 3 ml cercariae-free drinking water in glass tubes at approximately 24 °C. After 24 h, shell height, and the number of cercariae shed was counted on a grit by stereomicroscope (for 65% of snails duplo counts were made by one person, and the two counts were averaged), and cercariae species were identified according to standard keys (Ditrich et al., 1992; Schell, 1985; Scholz et al., 2000; Whitfield et al., 1975). Snails that did not shed cercariae were crushed and observed under stereomicroscope. If rediae or cercariae were present, this snail was recorded as false-negative.

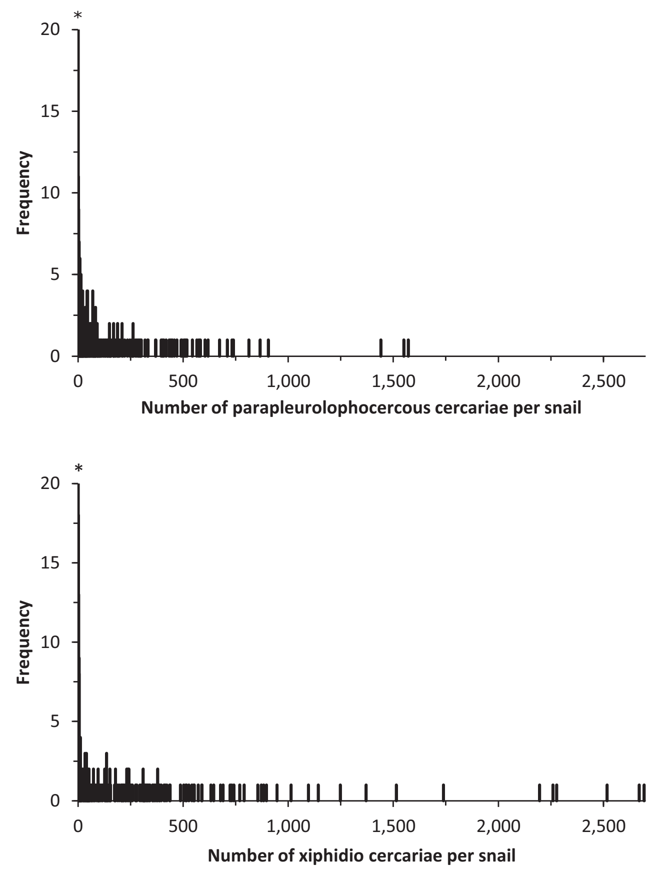

Fig. 1. Frequency of snails per parapleurolophocercous and xiphidio cercariae burden. The *y*-axes were truncated: 4854 snails did not shed cercariae.

Two prevalences were recorded: a snail was considered infected if (1) at least one cercariae was shed, (2) if cercariae were found after shedding or crushing. Cercariae burden was recorded after shedding only.

Statistical analyses were performed using SAS 9.2 (SAS Institute Inc., 2004). Infection with parapleurolophocercous or xiphidio cercariae in snails (yes/no) was analyzed with generalized linear models with a binomial distribution and a logit link function (i.e. logistic regression analysis) in which the variable land use, snail size and their interaction were included as explanatory variables. Linearity between logit and snail size was assessed as described by Dohoo et al. (2003). As snails within ponds and sampling areas nested within ponds cannot be regarded as independent observations, pond and area within pond were included as random effects using an exchangeable correlation structure. As area within pond was not significant, explained only 1.2% of non-explained variance for both cercariae types, and did not change *P*-values, it was excluded from the model. Results were expressed as prevalences and odds ratios.

An excess of zero counts (Fig. 1) leads to over-dispersion, which can be dealt with by using zero-inflation models. Such models