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Fasciola hepatica and *Paramphistomum daubneyi*: field observations on the transport and outcome of floating metacercariae in running water

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Abstract

Experimental investigations in eight open drainage ditches and furrows from central France were carried out to analyse the dispersal of floating metacercariae of two digenean species by running water and to determine the outcome of larvae which settled on *Nasturtium officinale* (watercress). The frequencies of larvae found after their transport by water ranged from 33% to 49.7%, thus indicating that more than half of the metacercariae used in this experiment had fallen to the bottom of the water during this transport. The nature of the site (furrow, or ditch supplied by a spring) had a significant effect on the distribution of floating larvae, while the digenean species had no effect. Low percentages of metacercariae on watercress were noted in furrows (3.5–4.3% of larvae) and ditches (0.8–1.3%). When the watercress grew, most larvae that had settled on leaves and stems died but there were always several living metacercariae on this plant (0.7–1.5% of larvae for *Fasciola hepatica* and 0.2–0.5% for *Paramphistomum daubneyi*). The weak buoyancy of these floating cysts on running water limited their dispersal and, consequently, led to a real diminution of risks incurred by definitive hosts towards these metacercariae.

Introduction

After their exit from the snail, cercariae of *Fasciola hepatica* swim freely in the water for a few minutes, settle on various objects using their ventral sucker, and encyst by secreting a cyst wall and losing their tail. Encystment may also take place upon the surface of the water and result in floating metacercariae, as they develop air-filled lacunae in their outer cyst wall (Esclaire *et al.*, 1989). The frequency of these larvae varies with the lymnaeid species used for experimental infections and also its shell height at miracidial exposure; for example, 1.6 to 17.7% of parasites which exit from the snail *Galba truncatula* form floating metacercariae (Vareille-Morel *et al.*, 1994; Bargues *et al.*, 1996). These larvae are able to develop into adult flukes in rabbits (Vareille-Morel *et al.*, 1993).

Despite several investigations performed over the past 12 years in central France, the finding of free-floating metacercariae of *F. hepatica* in the habitats of *G. truncatula*

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was unsuccessful. As the occurrence of human infections by ingestion of water containing metacercariae or by the consumption of salads contaminated with floating metacercariae was highly suspected in France (Cadel *et al.*, 1996) or in the Bolivian Altiplano (Hillyer & Apt, 1997), the result we have obtained in central France raises a fundamental question on the ability of such parasites to float upon the surface of the water and/or to be dispersed by running water. Vareille-Morel *et al.* (1993) showed that numerous floating metacercariae fell to the bottom of running water, so that a few cysts of *F. hepatica* stayed on watercress leaves. However, this experiment was carried out under laboratory conditions and it remains to be seen whether these results would be confirmed under natural conditions. Therefore, the two following questions arise: (i) what is the outcome of *F. hepatica* floating metacercariae when they are transported by running water; and (ii) might these results be generalized if the floating metacercariae of another digenean, *Paramphistomum daubneyi*, are used in the same experiment? In an attempt to answer these questions, two experiments were carried out in June–July 2001 in drainage ditches and furrows located in central France.

Materials and methods

The eight sites (four open drainage ditches and four furrows) studied were situated in four swampy meadows, on the communes of Bellac and Mézières sur Issoire, department of Haute Vienne. The lengths of these sites ranged from 40 to 55 m, and they were 45 cm wide, 15 cm deep, and had a 4–6% gradient. Water coming from permanent springs flowed in the four ditches, the flow ranging from 4.6 to 6.5 l min^{-1} with a velocity of 1.2 to 2.5 cm s^{-1} during the experiments in June and July. The four furrows received only rainfall and so had lower values for the flow (1–2 l min^{-1}) and velocity (0.3–0.5 cm s^{-1}) of the water. All these ditches and furrows were situated on underlying two-mica granite, flowed into different ponds, and did not have any phanerogamic vegetation at the time of the experiments. They were isolated from cattle by fencing to avoid any infection of these ruminants with metacercariae of *F. hepatica* and *P. daubneyi*. In each site, two operations were performed: (i) ten young plants of *Nasturtium officinale* (watercress) were placed in the water, at a distance of about 10 m from the pond; and (ii) an empty 5-m^3 tank was placed at the mouth of each ditch or each furrow to pump running water during each experiment for 30 min (after the last introduction of metacercariae into every channel) and to recover metacercariae via a filter (area: 1 m^2, pore size: 75 μm) before the progressive discharge of water in the pond.

A total of 2400 floating metacercariae for *F. hepatica* and 2400 for *P. daubneyi* were used in the present study. They originated from a total of 800 experimentally-infected snails (400 for each digenean species), belonging to three populations of *G. truncatula*. The technique used was that reported by Abrous *et al.* (1998). Each 4-mm high snail was exposed to two newly hatched miracidia of *F. hepatica* (or *P. daubneyi*) for 4 h and was raised in a breeding container at 20°C for 30 days before subsequently being placed in an individual 35-mm Petri dish with spring water and a piece of romaine lettuce. Every day, the floating metacercariae were collected from Petri dishes and stored in water at 4°C until their use in the field.

The first experiment (table 1) was performed to study the dispersion of floating metacercariae when transported by running water. A total of 1600 cysts of *F. hepatica* were introduced in a ditch (with a spring) and a furrow using four replicates. The protocol was the same for the 1600 cysts of *P. daubneyi*. The 200 metacercariae used for each ditch (or furrow) and each replicate were introduced by groups of 50 cysts each at the peripheral extremity of each ditch (or each furrow) and placed in the running water which carried them to the pond. The ditch (or furrow) was carefully investigated during the subsequent 12 h to collect (with a 75-μm sieve) and count (under a stereomicroscope) the floating metacercariae which (i) remained near the introduction point, (ii) stayed on the surface of the water, (iii) settled on the different parts of watercress plants (emerged sides of floating leaves, submerged leaves, roots, and stems, or buried roots), or (iv) were retained on the 75-μm filter. The parameters studied were the number of floating metacercariae in each of the aforementioned categories and the corresponding frequencies. A Chi-square test and a two-way analysis of variance (Stat-Itcf, 1988) were used to determine levels of significance.

In the second experiment (table 1), the outcome of floating metacercariae when they remained on watercress for 21 days was studied. Four hundred metacercariae of *F. hepatica* (8 groups of 50 cysts each) were introduced in

Table 1. The principal characteristics of experiments performed in the field with floating metacercariae of *Fasciola hepatica* and those of *Paramphistomum daubneyi*.

Aim of the experiment	Digenean species (and total number of metacercariae introduced)	Open drainage ditches or furrows	Number of replicates per ditch or furrow	Duration of each trial
Transport of metacercariae by running water	*Fasciola hepatica* (1600)	1 furrow, 1 ditch with spring	4, with 200 metacercariae per replicate	12 hours
	Paramphistomum daubneyi (1600)	1 furrow, 1 ditch with spring	4, with 200 metacercariae per replicate	12 hours
Outcome of metacercariae on watercress	*F. hepatica* (800)	1 furrow, 1 ditch with spring	A single replicate with 400 metacercariae	21 days
	P. daubneyi (800)	1 furrow, 1 ditch with spring	A single replicate with 400 metacercariae	21 days

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running water at the peripheral extremity of each ditch (or each furrow). The protocol was the same as described for metacercariae of *P. daubneyi*. Metacercarial counts were only performed on day 21 post-introduction by collecting watercress plants (they were 8–12 cm high at that time) and determining (under a stereomicroscope) the number of cysts on each of the following parts: upper sides of emerged leaves, lower sides of emerged leaves, emerged stems, or submerged roots. After counting, metacercariae were removed from the watercress and placed in Bouin's fixative for 48 h. Serial sections, 5 μm thick, were then made and stained with Harris' haematoxylin and modified Gabe's trichrome. A final histological examination determined if these parasites were living or dead. The parameters were the numbers of living metacercariae and of dead larvae (Vareille-Morel *et al.*, 1993) found on the different parts of watercress. A Chi-square test (Stat-Itcf, 1988) was then used to determine levels of significance.

Results

Transport of metacercariae by running water

The frequency of larvae found (table 2) ranged from 33% to 49.7%, indicating that more than half of metacercariae used in this experiment had fallen to the bottom of the water. The percentages recorded on watercress were very low: 3.5% and 4.3% in furrows, 0.8% and 1.3% in ditches. A comparison of these values using analysis of variance was performed with the number of metacercariae counted in the vicinity of the introduction points, on the surface of the water, and on the watercress. The nature of the site (ditch, or furrow) had a significant effect ($F = 6.11$, $P < 0.01$) on the distribution of floating metacercariae, while the digenean species had no effect.

Table 3 gives a more detailed distribution of metacercariae found on the different parts of *N. officinale*. The highest percentages were recorded on different submerged parts of the watercress, whatever the nature of the site and digenean species. However, there was no significant difference between these frequencies. Twenty percent (*F. hepatica*) and 21.4% (*P. daubneyi*) of metacercariae counted on the watercress from furrows were located on the emerged sides of floating leaves, whereas, in the case of ditches, no larvae were found on the corresponding parts of these leaves. An examination of watercress roots demonstrated the presence of metacercariae in 30–36.3% of ditches and furrows but no significant differences were found.

Table 2. The distribution of floating metacercariae of *Fasciola hepatica* and *Paramphistomum daubneyi* in relation to the different sections of ditches and furrows.

		Number of metacercariae found on different places				
Parasite	Ditch or furrow*	Introduction point	Surface of the water	Watercress (%)	75-μm filter	Total number (%)
Fasciola hepatica	Furrow	37	216	28 (3.5)	117	398 (49.7)
	Ditch	4	77	7 (0.8)	176	264 (33.0)
Paramphistomum daubneyi	Furrow	45	194	35 (4.3)	88	362 (45.2)
	Ditch	9	102	11 (1.3)	271	398 (49.1)

*Four replicates with a total of 800 metacercariae per ditch or furrow.

Table 3. The location of floating metacercariae of *Fasciola hepatica* and *Paramphistomum daubneyi* on the different parts of watercress, *Nasturtium officinale*.

			Metacercariae found on watercress	
Parasite	Ditch or furrow	Parts of watercress	Number	Frequency (%)*
Fasciola hepatica	Furrow	Emerged sides of floating leaves	6	21.4
		Submerged leaves, roots, and stems	14	50.0
		Buried roots	8	28.5
	Ditch	Emerged sides of floating leaves	0	0
		Submerged leaves, roots, and stems	5	71.4
		Buried roots	2	28.6
Paramphistomum daubneyi	Furrow	Emerged sides of floating leaves	7	20.0
		Submerged leaves, roots, and stems	21	60.0
		Buried roots	7	20.0
	Ditch	Emerged sides of floating leaves	0	0
		Submerged leaves, roots, and stems	7	63.6
		Buried roots	4	36.3

*Number of metacercariae counted on a part of watercress/total number of larvae recorded on *N. officinale* (see table 2).

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Table 4. The outcome of floating metacercariae of *Fasciola hepatica* and *Paramphistomum daubneyi* on the different parts of watercress, *Nasturtium officinale* on day 21 post-introduction.

Parts of watercress	Physiological state of cysts	Number of metacercariae			
		Fasciola hepatica		*Paramphistomum daubneyi*	
		Furrow	Ditch	Furrow	Ditch
Upper sides of emerged leaves	Living	0	0	1	0
	Dead	7	1	9	3
Lower sides of emerged leaves	Living	0	0	0	0
	Dead	11	6	16	8
Emerged stems	Living	1	0	0	1
	Dead	9	5	13	2
Submerged roots	Living	5	3	2	1
	Dead	17	26	19	25
Total number (% in relation to numbers of introduced metacercariae*)	Living	6 (1.5)	3 (0.7)	1 (0.2)	2 (0.5)
	Dead	44 (11.0)	38 (9.5)	57 (14.2)	38 (9.5)

*Four hundred metacercariae per ditch (or furrow) and per digenean species.

Outcome of metacercariae on watercress

During the 21 days of the second experiment, 23 mm of rain were recorded in the meadows located around Mézières sur Issoire, and 23 mm recorded from Bellac (data not shown). Table 4 gives the distribution of living or dead metacercariae for *F. hepatica* and *P. daubneyi* in relation to different parts of the watercress. The frequency of living metacercariae on day 21 post-introduction was very low: 0.7% and 1.5% for *F. hepatica*, 0.2% and 0.5% for *P. daubneyi*. By contrast, the percentages of dead metacercariae were higher: 9.5% and 11.0% for *F. hepatica*, 9.5% and 14.2% for *P. daubneyi*. No significant differences between these frequencies were noted for each type of larvae (living, or dead) considered separately, whatever the mode of comparison.

Discussion

The presents results agree with those of Vareille-Morel *et al.* (1993) on floating metacercariae of *F. hepatica* under laboratory conditions. In the two studies, transport of such larvae by running water induced the elimination of numerous metacercariae: 68.6% for Vareille-Morel *et al.* (1993), and 50.3 and 67%, respectively, in the ditches and furrows used in the present study. Moreover, the number of larvae settling on *N. officinale* remained low (6.9% for Vareille-Morel *et al.* in 1993, 0.8 and 3.5% in the present study) and most of them died when the watercress grew. Under these conditions, one might wonder whether this larval stage was useful in the transmission of fascioliasis, as these metacercariae could float for over three months on the surface of stagnant water (Esclaire *et al.*, 1989) but were not of great importance in the dispersal of the parasite by running water. Consequently, the infection of cattle or sheep with these metacercariae would be at its maximum in small places which had stagnant water during the greatest part of the year, as often occurred in the swampy meadows of central France. By contrast, if the formation of these metacercariae occurred in running water, most of them would rapidly fall to the bottom of the water, thus leading to a real diminution of risks incurred by potential definitive hosts.

Despite several slight differences in the frequency of floating metacercariae along ditches and furrows, the results obtained for the distribution of *P. daubneyi* larvae were close to those obtained with *F. hepatica*. This finding suggested that the digenean species used in this study (*F. hepatica* or *P. daubneyi*) did not affect the distribution of these metacercariae when transported by running water. To explain this last result, two perhaps complementary hypotheses might be proposed. The first was to consider that the collars (Esclaire *et al.*, 1989) surrounding each floating metacercaria of *F. hepatica* and each cyst of *P. daubneyi* would be very similar in their structure. Another would be to explain the weak buoyancy of floating metacercariae on running water by the small size of the collars (a mean width of 51.1 μm: Esclaire *et al.*, 1989), despite the presence of air-filled lacunae in these structures.

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