Journal of Helminthology (2001) **75,** 307–311 DOI: 10.1079/JOH200159

Metacercarial aggregation in Digenea (*Fasciola hepatica* and *Paramphistomum daubneyi*): environmental or species determinism?

M. Abrous[1,2], C. Vareille-Morel[3], D. Rondelaud[1]*, G. Dreyfuss[2] and J. Cabaret[3]

[1]Laboratoire d'Histopathologie Parasitaire, Faculté de Médecine, and [2]Laboratoire de Parasitologie, Faculté de Pharmacie, 2, rue du Docteur Raymond Marcland, 87025 Limoges Cedex, France: [3]Laboratoire de Malacologie Appliquée, Faculté des Sciences, 123, avenue Albert Thomas, 87060 Limoges Cedex, France: [4]Station de Pathologie Aviaire et de Parasitologie, Institut National de la Recherche Agronomique, 37380 Nouzilly Cedex, France

Abstract

Metacercarial aggregation of *Fasciola hepatica* and *Paramphistomum daubneyi* was studied under experimental conditions to determine if the formation of these aggregates was influenced by environmental factors, or it was a characteristic of trematode species. This process was studied using the confinement of infected snails on the bottom of Petri dishes (diameter, 14 cm) for 3 days. The formation of metacercarial aggregates of *F. hepatica* was not significantly modified by environmental factors such as intensity and duration of lighting, quality and volume of water. Metacercariae of *F. hepatica* were more numerous on the Petri dish walls and 63.9% of them constituted aggregates. In contrast, most metacercariae of *P. daubneyi* were found on the Petri dish bottoms and 78.3% of them were isolated or in groups of two metacercariae each. The mean number of metacercariae per aggregate ranged from 6.7 to 12.2 in the case of *F. hepatica*, and from 2.7 to 4.5 in the case of *P. daubneyi*. However, these mean numbers were independent of the site of cercarial attachment. The tendency of cercariae to form metacercarial aggregations was a characteristic of *F. hepatica* and was species determined.

Introduction

Parasites are generally found aggregated among hosts (Anderson & Gordon, 1982). As has been shown theoretically, the features of parasite aggregation may have important consequences for the population dynamics of host–parasite interactions (May, 1977). An equilibrium state between host and parasite is possible when aggregation is not too intense. When the equilibrium does not exist, the number of parasites per host increases with increasing aggregation when aggregation is small, and is roughly independent of aggregation when aggregation is large. Parasite aggregation plays then an important role on the infection rate of the host and its study might explain the difference of infection between parasite species. The ratio of prevalence in ruminant faeces (definitive host) to prevalence in *Lymnaea truncatula* (intermediate host) was always higher in *Fasciola hepatica* infections than in *Paramphistomum daubneyi* (7.3 versus 4.1 in cattle from four farms, and 4.1

*Author for correspondence
Fax: 33 5 55 43 58 93
E-mail: rondelaud@pharma.unilim.fr

vs 1.8 in sheep from three farms), as calculated from the data of Abrous *et al.* (1999a). This means that fewer infected *L. truncatula* are needed to infect ruminants with *F. hepatica* than *P. daubneyi*. This might be due in part to the fact that *F. hepatica* metacercariae are often found aggregated in the field (Roberts, 1950; Taylor, 1965; Pecheur, 1967, 1971). No information is available for *P. daubneyi*. The metacercarial aggregation in *F. hepatica* might be then due either to physical influences of environment, or to an intrinsic response related to species adaptation. We intend to experimentally investigate the following hypotheses: (i) which environmental factor could influence metacercarial aggregation in *F. hepatica*; and (ii) is metacercarial aggregation common to both digeneans, or is it a characteristic of *F. hepatica* only?

Materials and methods

The population of *L. truncatula* occurred in ditches along road D20, in the commune of Migné, department of Indre, central France. Snails measuring 4 ± 0.1 mm in height were collected from this site in April and May 1997. This colony was known to be devoid of natural trematode infections because monthly samples of 50 adult snails each over 2 years revealed no larval forms in dissected snails. A total of 1700 preadult snails, 4 ± 0.1 mm high, were collected from this population, transported to the laboratory under constant conditions, and kept for 48 h at room temperature (20°C) before the start of the experiment. To obtain eggs we collected adult *P. daubneyi* from the rumen of cattle and placed them in a normal saline solution (0.9% NaCl, 0.45% glucose) for 4 h at 40°C. The eggs of *F. hepatica* were collected from the gallbladders of heavily infected cattle. Eggs of both trematodes were incubated in spring water at 20°C for 20 days in complete darkness prior to hatching of miracidia under artificial light.

Snails were divided into two groups, with 1450 snails from the first group being individually exposed to two miracidia of *F. hepatica*. A similar protocol was used for the other group (250 snails) but each of these snails was exposed to two miracidia of *P. daubneyi*. Snails from both groups were reared until day 35 post-exposure in standard breeding containers (five snails per litre of water) that were placed in an air-conditioned room at 20°C. At day 35, infected snails were detected under the stereomicroscope by the presence of larval forms within the snail body, under the transparent shell. Infected snails were then isolated in 14-cm diameter Petri dishes to study cercarial shedding.

The first experiment was performed to study the influence of environmental factors, such as light, quality of water, or water disturbance, on the metacercarial aggregation of *F. hepatica*. Fifty infected snails were used as controls. Each snail was confined to an ovoid and transparent container (height, 1.5 cm; section surface, 0.78 cm^2) perforated by fifteen 2-mm diameter holes and supported by a bracket in a central position in the base of a Petri dish (fig. 1). A piece of lettuce was added to the perforated container and 60 ml of water added to the Petri dish. The dishes were maintained in an air-conditioned room, at 20°C, under artificial lighting of 12 h duration (0700–1900) for an intensity of 3000 lux.

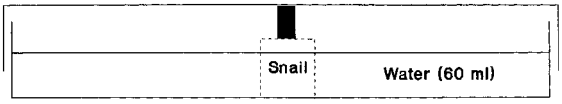

Fig. 1. Petri dish (diameter, 14 cm) and perforated container (----) used to study the metacercarial aggregates of *Fasciola hepatica* and of *Paramphistomum daubneyi*. (■, Angular support made of wood.

Eighteen groups each comprising 50 infected snails, were used to study the influence of factors as follows: (i) the position of the perforated container containing the infected snail in the Petri dish (along the dish wall, at 3.5 cm from the wall, or in the centre of the dish bottom); (ii) the intensity of artificial lighting on the recipient (1000, 2000, 3000, or 4000 lux); (iii) the duration of lighting (6, 9, 12, or 15 h); (iv) the quality of water (1 CO_2-bubble per min, 1 air-bubble per min, or no bubbling); (v) the quantity of water in the Petri dish (20, 40, 60, or 80 ml); (vi) the number of water changes throughout the experiment (2 per day, 1 per day, only at day 2, or no change); and (vii) the frequency of water stirring (1 stir per min, 1 per 15 min, or no stir).

A second experiment for the study of metacercarial aggregates was performed using 157 *L. truncatula* infected by *F. hepatica* and 171 snails infected by *P. daubneyi*. The protocol was similar to that of controls used in the first experiment. Cercarial shedding of each infected snail was studied for a 3-day period. The water in the Petri dish was changed daily. In the *F. hepatica* group, the water temperature after changing was the same (20°C), whereas in the *P. daubneyi* group it was 6–8°C and increased at 20°C in the subsequent hour (Abrous *et al.*, 1999b). Metacercariae were not removed from the Petri dishes during the 3-day period, so that aggregates and non-aggregated metacercariae were counted only on day 4 before the liberation of the snail from its perforated container.

Aggregates consisted of three or more metacercarial cysts, separated from each other by a distance of 200 μm or less. Groups of two metacercariae were considered as non-aggregated in this work, as their formation might be a random process. The first three parameters studied were the number of metacercarial aggregates, that of non-aggregated metacercariae, and the location of metacercariae in the different sites (dish walls, dish bottoms, water surface) of the Petri dishes. This location was determined using four quadrants for the Petri dish walls, four for the dish bottom, and four on the surface of the water. Other parameters were the number of metacercariae into each metacercarial aggregate, the density of aggregated metacercariae, and that of non-aggregated ones for each 1-cm^2 area of substrate. The frequency of aggregated metacercariae and that of non-aggregated ones in each site of the Petri dish were calculated in relation to the total number of metacercariae shed by infected snails. Analysis of variance and calculation of confidence intervals (CI, $P = 0.95$) were standard.

Results

Table 1 gives the numbers of aggregates of *F. hepatica*

https://doi.org/10.1017/S0022149X00701477 Published online by Cambridge University Press

Table 1. Distribution of aggregates of *Fasciola hepatica* metacercariae on the Petri dish walls in relation to the factors studied. Other metacercarial groups located in other sites of recipients are not considered in this table.

Factor studied (50 infected snails per group)	No. of metacercarial aggregates in quadrant no.				Significance (ANOVA)
	1	2	3	4	
Controls	16	19	14	17	–
Position of the container					
Along the dish wall	18	20	13	13	NS
At 3.5 cm from the wall	14	17	15	15	NS
Intensity of lighting					
1000 lux	15	13	15	17	NS
2000 lux	11	17	15	12	NS
4000 lux	16	13	12	16	NS
Duration of lighting					
6 h per day	18	15	12	13	NS
9 h per day	16	14	16	15	NS
15 h per day	11	14	11	17	NS
Quality of water					
CO_2	18	16	19	13	NS
Air	14	11	13	15	NS
Quantity of water per dish					
20 ml	13	14	15	19	NS
40 ml	11	17	12	13	NS
80 ml	13	13	16	14	NS
Number of water changes					
2 per day	7	5	8	9	$P < 0.01$
At day 2	17	13	16	14	NS
No change	18	12	12	17	NS
Stirring of water					
1 stir per min	14	11	15	14	NS
1 stir per 15 min	17	13	12	14	NS

Abbreviations: NS, non-significant difference; *P*, probability.

metacercariae recorded in the four quadrants of dish walls in relation to the different factors studied. When water was changed twice daily, the number of metacercariae was significantly lower than that recorded in controls ($F = 13.26$; $P < 0.01$). The other differences were not significant. Similar findings were also noted for the number of metacercarial groups found on the dish bottoms, or at the surface of the water (data not shown).

Table 2 gives the characteristics of metacercarial aggregates in relation to their location in Petri dishes. In the case of *F. hepatica*, the frequency of aggregated metacercariae was significantly higher than that of non-aggregated ones, on the Petri dish walls ($P < 0.001$) and on the water surface ($P < 0.001$). In the case of *P. daubneyi*, the number of non-aggregated metacercariae on the dish bottoms was significantly higher ($P < 0.01$)

Table 2. *Fasciola hepatica* and *Paramphistomum daubneyi*: the frequency of non aggregated and aggregated metacercariae, and the number of metacercariae per aggregate in relation to their location in Petri dishes.

Trematode	Parameter studied	Location of metacercariae in Petri dishes		
		Walls	Bottom	Water surface
Fasciola hepatica (n = 3624)	Frequency (%) ± CI 95*:			
	Non-aggregated metacercariae	16.9 ± 1.2	1.2 ± 0.3	2.3 ± 0.5
	Aggregated metacercariae	63.9 ± 1.6	1.2 ± 0.3	5.3 ± 0.7
	No. of aggregates	190	11	28
	No. of metacercariae per aggregate (mean ± S.D.)	12.2 ± 4.5	6.5 ± 2.1	6.7 ± 3.1
Paramphistomum daubneyi (n = 2956)	Frequency (%) ± CI 95*:			
	Non-aggregated metacercariae	3.4 ± 0.7	78.3 ± 1.5	1.4 ± 0.4
	Aggregated metacercariae	1.9 ± 0.5	5.8 ± 0.8	0,7 ± 0.9
	No. of aggregates	4	21	5
	No. of metacercariae per aggregate (mean ± S.D.)	4.5 ± 1.8	3.5 ± 1.7	2.7 ± 2.1

* CI 95 (confidence interval at probability 0.95).
n = total number of metacercariae shed by infected snails during 3 days (*F. hepatica*: 157 snails, *P. daubneyi*: 171 snails).

https://doi.org/10.1017/S0022149X00701477 Published online by Cambridge University Press

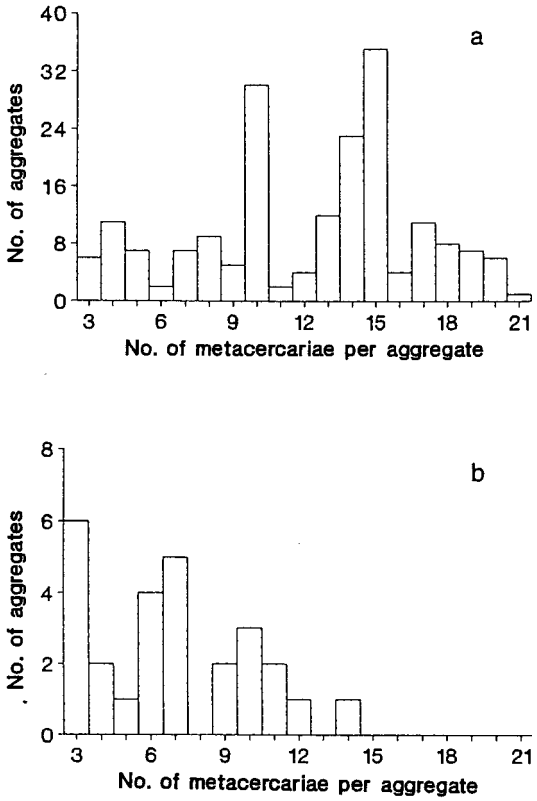

Fig. 2. The distribution of *Fasciola hepatica* aggregates in relation to the number of metacercariae per aggregate (a) on the walls of the Petri dish and (b) on the water surface.

than that of aggregated ones. The mean number of metacercariae per aggregate ranged from 6.7 to 12.2 in the case of *F. hepatica*, although the difference between the mean values recorded in these different locations was not significant. The mean figures were lower in the case of *P. daubneyi* (2.7 to 4.5) and no significant difference between the mean values was found. However, in each of the sites considered in the Petri dishes, the number of metacercariae per aggregate was significantly higher for *F. hepatica* than for *P. daubneyi* (dish walls: $F = 9.29$, $P < 0.001$; dish bottoms: $F = 4.63$, $P < 0.05$; water surface: $F = 4.45$, $P < 0.05$).

Figure 2 shows the distribution of metacercarial aggregates of *F. hepatica* in relation to the number of metacercariae per group. On the dish walls of Petri dishes (fig. 2a), the number of metacercariae per aggregate ranged from 3 to 21, and two peaks were found at 10 and 15 metacercariae per group, respectively. When the metacercarial aggregates were floating on the water surface (fig. 2b), the range of metacercariae per group was 3–14, and the number of aggregates tended to decrease with an increasing number of metacercariae per group. A similar finding was noted in the case of *P. daubneyi*, whatever the site considered in the Petri dishes. However, the number of metacercariae per aggregate ranged only from 3 to 6 (data not shown).

Calculation of metacercarial densities into each site of the Petri dish was performed using the surface of the dish walls (132 cm^2), bottom (615 cm^2), and water surface (615 cm^2). This evaluation by available surfaces was needed, as there were large differences in the surface between dish walls, dish bottoms, and the water surface. In the case of *F. hepatica*, the respective densities of non-aggregated metacercariae were 4.60, 0.20, and 0.13 per cm^2 of site, while those of aggregated metacercariae were 17.5, 0.08, and 0.31 per cm^2, respectively. In the case of *P. daubneyi*, the respective densities were 0.76, 3.76, and 0.06 per cm^2 of site for non-aggregated metacercariae, and 0.42, 0.27, and 0.03 per cm^2 for aggregated metacercariae (data not shown).

Discussion

Metacercarial aggregates were more numerous for *F. hepatica* than for *P. daubneyi*. This finding clearly demonstrated that the formation of metacercarial aggregates was a characteristic of *F. hepatica* and did not depend on the isolate of this trematode used for these experiments, as their presence had already been noted since 1989 in some studies on the cercarial shedding of *F. hepatica*, whatever the nature of the definitive host (cattle, sheep or rabbit) from which trematode eggs were collected (Dreyfuss & Rondelaud, 1994; Rondelaud & Dreyfuss, 1995, 1997). Conversely, in the case of *P. daubneyi*, the high frequency of non-aggregated metacercariae found in this experiment was in agreement with the report by Abrous *et al.* (1999b) on cercarial shedding of this trematode.

The first advantage of metacercarial aggregation was to concentrate parasites, thus permitting a better chance to infect the mammalian host (Anderson & May, 1985). However, the utility of this advantage was open to question, for parasite aggregation had several different influences on the success of transmission. First, the populations of parasite and host reach an equilibrium when aggregation is not too important, and the equilibrium will be maintained when aggregation remains modest (May, 1977). Secondly, the number of parasites per host increases as aggregation rises, mostly when aggregation is not too high. Thirdly, the prevalence of infection increases with an increasing number of exposures, but there again the distribution of infective material is of high importance (Anderson, 1978). According to the latter author, prevalence will be higher if the distribution fits a positive binomial (more even than random one) or Poisson (random) than negative binomial (aggregated). Slight aggregations may result in similar prevalences to random or less than random distributions, whereas highly aggregated distributions resulted in poor prevalence values. As the metacercarial aggregation in *F. hepatica* was much higher than in *P. daubneyi*, it should result in a stable equilibrium between host and parasite (bearing in mind that *F. hepatica* is much more pathogenic than *P. daubneyi*), the number of parasites per host could be maintained at a tolerable level, and the prevalence of infection could reach its best tolerable level in the host population.

The second advantage of metacercarial aggregation might be a better conservation of moist conditions in aggregated metacercariae, thus favouring parasite survival

https://doi.org/10.1017/S0022149X00701477 Published online by Cambridge University Press

under field conditions. As the encystment of most *F. hepatica* cercariae occurred on the water surface and underlying zone (88.4% of metacercariae on water surface and dish walls vs. 84.1% of *P. daubneyi* metacercariae on dish bottoms, see table 2), metacercarial aggregation might avoid a too rapid dehydration of parasites and their subsequent death when stagnant water disappeared from the snail habitats in summer. To verify this last proposition, it would be useful to measure the duration of evaporation in metacercarial aggregates in relation to their size and, afterwards, to test metacercarial viability by studying their excystment under *in vitro* conditions.

References

Abrous, M., Rondelaud, D., Dreyfuss, G. & Cabaret, J. (1999a) Infection of *Lymnaea truncatula* and *Lymnaea glabra* by *Fasciola hepatica* and *Paramphistomum daubneyi* in farms of central France. *Veterinary Research* **30**, 113–118.

Abrous, M., Rondelaud, D. & Dreyfuss, G. (1999b) Influence of low temperatures on the cercarial shedding of *Paramphistomum daubneyi* from the snail *Lymnaea truncatula*. *Parasite* **6**, 85–88.

Anderson, R.M. (1978) Population dynamics of snail infection by miracidia. *Parasitology* **77**, 201–224.

Anderson, R.M. & Gordon, D.M. (1982) Processes influencing the distribution of parasite numbers within host populations with special emphasis on parasite-induced host mortalities. *Parasitology* **85**, 373–398.

Anderson, R.M. & May, R.M. (1985) Helminth infections of humans: mathematical models, population dynamics, and control. *Advances in Parasitology* **24**, 1–101.

Dreyfuss, G. & Rondelaud, D. (1994) *Fasciola hepatica*: a study on the shedding of cercariae from *Lymnaea truncatula* raised under constant conditions of temperature and photoperiod. *Parasite* **1**, 401–404.

May, R.M. (1977) Dynamical aspects of host-parasite associations: Crofton's model revisited. *Parasitology* **75**, 259–276.

Pecheur, M. (1967) La cercaire de *Fasciola hepatica*. Le rôle de la couleur, de la lumière et des plantes sur le choix de l'endroit de fixation. La cercaire est-elle infestante? *Annales de Médecine Vétérinaire* **6**, 349–355.

Pecheur, M. (1971) A few considerations on the cercariae and metacercariae of *Fasciola hepatica*. *Proceedings, Second International Liverfluke Colloquium, Wageningen, 1967* 148–149.

Roberts, E.W. (1950) Studies on the life-cycle of *Fasciola hepatica* (Linnaeus) and of its snail host, *Limnaea* (*Galba*) *truncatula* Müller in the field and under controlled conditions. *Annals of Tropical Medicine and Parasitology* **44**, 187–206.

Rondelaud, D. & Dreyfuss, G. (1995) *Fasciola hepatica*: the influence of the definitive host on the characteristics of infection in the snail *Lymnaea truncatula*. *Parasite* **2**, 275–280.

Rondelaud, D. & Dreyfuss, G. (1997) Variability of *Fasciola* infection in *Lymnaea truncatula* as a function of snail generation and snail activity. *Journal of Helminthology* **71**, 161–166.

Taylor, E.L. (1965) Fascioliasis and the liver-fluke. 235 pp. FAO Agricultural Studies, no. 64.

(Accepted 10 January 2001)