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Larval development of *Fasciola hepatica* in experimental infections: variations with populations of *Lymnaea truncatula*

P. Vignoles, G. Dreyfuss and D. Rondelaud*

UPRES EA no. 3174, Facultés de Pharmacie et de Médecine, 2 rue du Docteur Raymond Marcland, 87025 Limoges Cedex, France

Abstract

A retrospective study was undertaken on 70 French populations of *Lymnaea truncatula* experimentally infected with *Fasciola hepatica* to determine whether or not susceptibility of snails to infection influenced redial and cercarial production. Results were compared with those obtained from two control populations, known for prevalences higher than 60% when experimentally infected with *F. hepatica*. In the 70 other populations examined, the prevalences ranged from 2 to 75%. In 55 of these populations, where the prevalence was more than 20%, a high proportion (50.1–56.8%) of snails died after cercarial shedding, whereas in the other groups (non-shedding snails with the most differentiated larvae being free cercariae, rediae containing cercariae, immature rediae, or sporocysts, respectively), snail death was significantly less. In 11 populations, where the prevalence values were 5–19%, only 14% of snails died after cercarial shedding, whereas snails with free cercariae, rediae with cercariae, or immature rediae showed significant increases in snail mortality. In the remaining four snail populations, with prevalences of less than 5%, the most differentiated larval forms were only immature rediae and/or sporocysts. Overall, the number of rediae containing cercariae significantly decreased with decreasing prevalence values. The low prevalence of experimental infection in several populations of snails might be explained by the occurrence of natural infections with miracidia originating from a mammalian host other than cattle, and/or by genetic variability in the susceptibility of snails to infection.

Introduction

The susceptibility of the snail *Lymnaea truncatula* to *Fasciola hepatica* infections depends on several factors and the most known are environmental. The prevalence of natural infections in snails is considerably higher during years with a high precipitation (Gracyzk & Fried, 1999). In most countries, the transmission of *F. hepatica* by snails shows a strong seasonal pattern and mainly occurs in the spring and/or summer generations of *L. truncatula* (Roberts & Suhardono, 1996: Rondelaud & Dreyfuss, 1997). Several biotic factors, such as the population of *L. truncatula* (Boray, 1978) or the definitive host from which the eggs of *F. hepatica* (Rondelaud & Dreyfuss, 1995) originated, may also influence the success of *F. hepatica* infecting snails. In a susceptible population of *L. truncatula*, experimental infections with miracidia which hatched from eggs after their collection in cattle induced low snail mortalities, high infection rates, and the production of numerous cercariae. In contrast, if the population is not very susceptible to *Fasciola* infections, snail mortalities are high, whereas infected snails are few in number and shed only a small proportion of cercariae (Rondelaud, 1993).

Variability in snail infections with *F. hepatica* might be explained by the frequency of previous natural encounters between the snail population and the parasite (Rondelaud, 1993). However, this parameter is not the only factor to influence the prevalence of infection in *L. truncatula*, as different snail populations occupying the

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

same habitat, such as swampy meadows, present variable results when experimentally infected with *F. hepatica* (Rondelaud & Dreyfuss, 1996). In view of this, two questions arise: (i) do all snails from the same population sustain full larval development of *F. hepatica* (with cercarial shedding) following penetration of the miracidium? and (ii) what are the effects on redial and cercarial production? To answer these questions, a retrospective study was performed on the results we have obtained over a 20-year period with experimental infections of *L. truncatula* with *F. hepatica*.

Materials and methods

The 72 populations of *L. truncatula* inhabited granite soil, in the departments of Corrèze, Creuse, and Haute Vienne (central France). These colonies were known to be devoid of any natural trematode infections because monthly samples of 50 adult snails each over two years revealed no larval forms in dissected snails. The calcium ion content in the water ranged from 7 to 28 mg l^{-1}, so that the shell height of adult snails scarcely reached 8 mm. The habitats of all snail populations were located near the terminal extremities of open drainage furrows in 72 different swampy meadows grazed by cattle. Samples of 100 snails each, measuring 4 mm in height and belonging to the spring generation, were collected from each habitat in April or May and were progressively acclimatized to a temperature of 20°C for a 48-h period. Eggs of *F. hepatica*, which originated from local cattle slaughtered at Limoges, were collected from the gallbladders of heavily infected animals and incubated for 20 days at 20°C in complete darkness prior to hatching of miracidia under artificial lighting (Ollerenshaw, 1971).

A total of 17 experiments with exposures of *L. truncatula* to *F. hepatica* miracidia were performed over the past 20 years. The first two lymnaeid populations (Blanzac, and Landouge, department of Haute Vienne) were used as controls, as prevalences higher than 60% were regularly noted in these snails when experimentally infected with miracidia (two per snail) of *F. hepatica* of cattle origin. Each experiment was performed using 100 snails from a control population and three or four samples (100 snails each) originating from the remainder of the populations of *L. truncatula* (one sample per population). Each snail was exposed to two newly hatched miracidia for 4 h. Snails were then reared for 30 days in polypropylene boxes 1 m by 55 cm and 15 cm high (50 snails per box). Each box contained small stones, with a 2-cm deep layer of water, which was constantly aerated. Water from the original site was added weekly to replace water lost by evaporation. Snails were fed with lettuce. These boxes were placed in an air-conditioned room under the following conditions: a constant temperature of 20°C, a diurnal photophase of 12 h with a 3000–4000 lux light intensity. At day 30 post-exposure (p.e.), surviving snails were individually placed in 35-mm diameter Petri dishes, each containing 2–3 ml of spring water and a piece of lettuce. These dishes were maintained in the same air-conditioned room at 20°C as the breeding boxes. Every day the water in the dishes was changed until the snails died. Cercariae were counted and removed from Petri dishes. A routine post-mortem dissection of snails that died after day 30 p.e. was performed to identify and count the most differentiated larval forms (sporocysts, immature rediae, rediae with cercariae, or free cercariae) present.

Survival rates of snails at day 30 p.e. were more than 60%. Infected snails, harbouring larval forms of *F. hepatica*, were classified, according to the length of the shedding period and the most differentiated larvae present, into the following groups: snails that died after a cercarial shedding (group 1); and non-shedding snails whose most differentiated larvae were free cercariae (group 2), rediae containing cercariae (group 3), sporocysts and immature rediae (group 4), or sporocysts (group 5), respectively. Parameters studied were the prevalence of experimental infection with *F. hepatica* and the frequency of infected snails in each group. The prevalence values were calculated using the ratio between the number of dead snails harbouring larval forms of *F. hepatica* and the number of snails surviving at day 30 p.e. The frequency values were determined in relation to the number of all infected snails in each population of *L. truncatula*. Other parameters measured were the number of rediae containing cercariae within each infected snail, the number of immature rediae, and free cercariae within snails, and the number of cercariae shed by each snail. The mean values and standard deviations were calculated, taking into account the following classes for the prevalences of infections: more than 60%, between 40 and 59%, between 20 and 39%, between 5 and 19%, and less than 5%. Comparison tests of experimental frequencies and one-way analysis of variance (Stat Itcf, 1988) were used to establish levels of significance.

Results

Figure 1 shows the prevalences of experimental infections recorded in the two populations of *L. truncatula* used as controls over the last 20 years. For each population, the prevalences were within the same range, i.e. from 68 to 79% for the population from Blanzac, and from 64 to 74% for the population from Landouge. No significant differences were recorded between prevalences in each population.

In the remaining 70 snail populations, the prevalences of experimental infections ranged from 2 to 75%. When the prevalence values (table 1) were more than 20% (55 populations of *L. truncatula*), the highest frequencies (50.1–56.8%) were those of snails which died after a cercarial shedding, whereas those from the four remaining other groups were clearly lower. Significant differences were recorded in the >60% class ($F = 21.70$, $P < 0.001$), the 40–59% class ($F = 15.01$, $P < 0.001$) and also in the 20–39% class ($F = 10.26$, $P < 0.001$). However, in each group of infected snails, differences between the frequencies recorded in these three classes of prevalences were not significant. In contrast, much variation in frequency was noted for prevalences of infections lower than 20%. In the 5–19% class (11 populations), the frequency of snail death after cercarial shedding was only 14%, while in snails containing free cercariae, rediae containing cercariae, or immature rediae, frequencies

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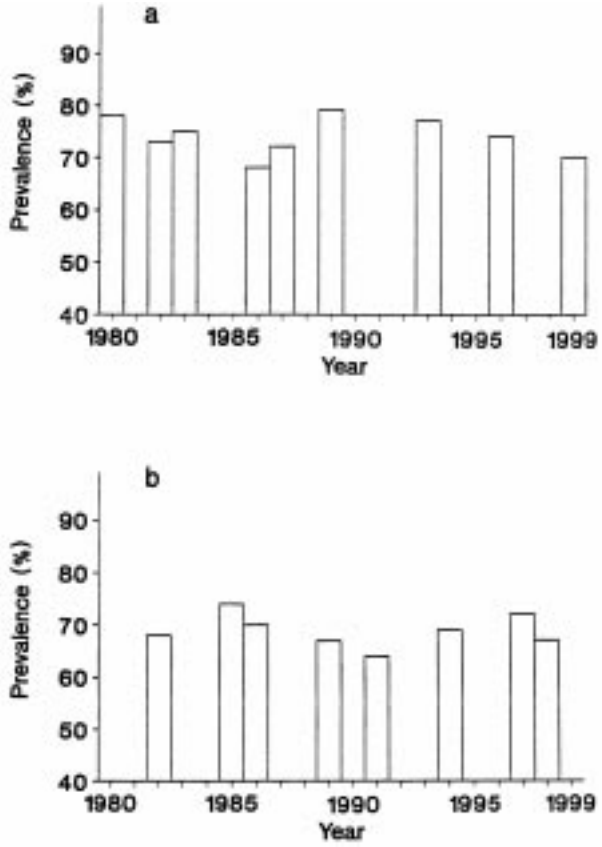

Fig. 1. Prevalences of experimental infections with *Fasciola hepatica* in two populations of *Lymnaea truncatula* (used as controls) over the past 20 years, in (a) Blanzac, and (b) Landouge.

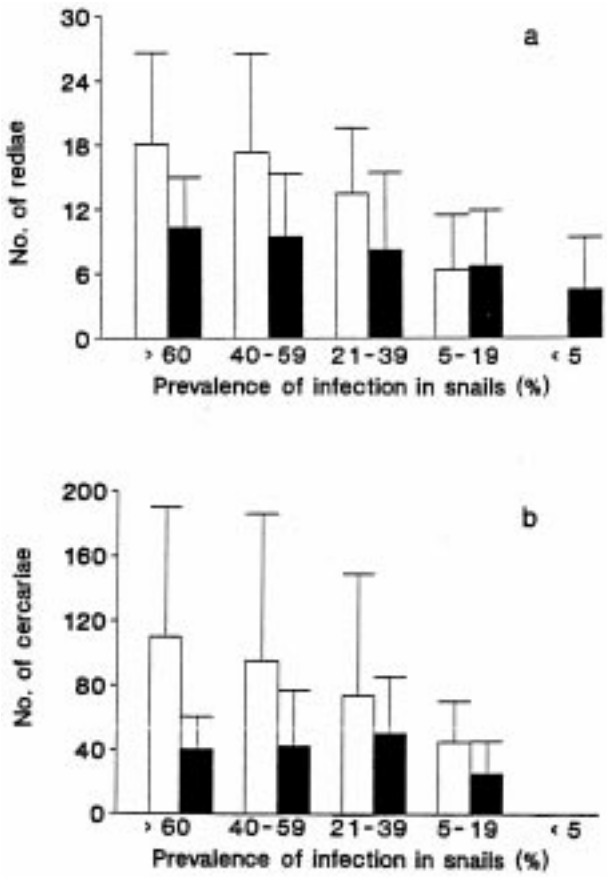

Fig. 2. Larval stages of *Fasciola hepatica* in 70 populations of *Lymnaea truncatula* relative to the five different prevalence classes observed for experimental infections. (a), the number of rediae containing cercariae (□) and that of immature, free rediae (■). (b), the number of cercariae released by infected snails (□) and the number of free cercariae present in dead snails (■).

were shown to increase. A significant difference between the former and the latter frequencies ($F = 3.57$, $P < 0.05$) was noted in this class of prevalences. In the <5% class (4 populations), only two groups of infected snails were found and no significant difference was noted.

Figure 2 shows redial and cercarial burdens in relation to the five classes defined for the prevalence of experimental infections. The number of rediae containing cercariae significantly decreased ($F = 2.92$, $P < 0.05$) with decreasing prevalence values, whereas the number of immature rediae showed no significant difference. In the <5% class, snails did not produce cercariae, while variation in cercarial numbers in the other prevalence classes were not significant.

Table 1. Mean values (±SD) for each group of *Lymnaea truncatula* infected with *Fasciola hepatica* (70 snail populations) relative to (i) length of cercarial shedding, or its absence, (ii) the most differentiated larval forms present in these snails, and (iii) the prevalences of experimental infections.

Prevalence of *Fasciola hepatica* infections (and number of snail populations)	Mean frequency (±SD) for each group of infected snails (in %)				
	Cercarial shedding	No shedding but free cercariae present	No shedding but rediae containing cercariae present	Immature rediae and sporocysts only	Sporocysts only
>60% (17)	56.8 ± 15.6	18.6 ± 8.3	10.7 ± 5.2	8.4 ± 4.5	5.5 ± 3.7
40–59% (15)	53.5 ± 18.3	21.1 ± 5.4	10.2 ± 6.3	6.0 ± 3.8	9.2 ± 5.4
20–39% (23)	50.1 ± 14.5	18.1 ± 9.5	14.2 ± 8.8	9.3 ± 11.5	10.3 ± 6.5
5–19% (11)	14.0 ± 11.5	35.3 ± 12.1	22.7 ± 11.2	19.5 ± 11.1	8.5 ± 7.6
<5% (4)	0	0	0	61.3 ± 35.5	38.7 ± 21.6

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Discussion

Prevalences of experimental infections in *L. truncatula* using two miracidia per snail showed variations in relation to the snail population under investigation. These variations could not be explained by the infectivity of different strains of *F. hepatica* miracidia, as the prevalence of infection recorded in each *L. truncatula* population used as controls over the past 20 years were similar, whatever the date of experimental infection (see fig. 1). As conditions of miracidial exposure and snail breeding were similar for the 70 populations studied, the differing levels of snail susceptibility to *F. hepatica* are likely to be related to the origin of the *L. truncatula* populations. The first explanation would be to consider the location of snail habitats and, consequently, that of populations in the meadows, as the prevalences of *F. hepatica* infections were higher in snails living at the extremity of each drainage furrow than in those colonizing the lower part of drainage ditches or river banks (Rondelaud & Dreyfuss, 1996). However, this first hypothesis is unlikely, as all populations of *L. truncatula* originated from habitats located at the periphery of open drainage networks in swampy meadows. Therefore, two perhaps complementary hypotheses might be proposed. The first is to assume that some populations of *L. truncatula* with low prevalences of experimental infections would not be adapted to miracidia originating from eggs collected from cattle and would have a well established relationship with miracidia originating from other mammalian sources. This first hypothesis is based on the fact that many wild rabbits and hares infected with *F. hepatica* were found to occur in or around these meadows in the Limousin region (Rondelaud *et al.*, 2001) and larval development of *F. hepatica* from eggs originating from these lagomorphs was complete, despite a low production of metacercariae (Rondelaud & Dreyfuss, 1995). The second hypothesis would be to explain these fluctuations in prevalences by genetic variability within *L. truncatula* populations in central France, although Trouvé *et al.* (2000) demonstrated that the level of this variability was low in this snail species.

Apart from the four populations in the <5% class, cercaria-shedding and non-cercaria-shedding snails were found in the 66 other populations of *L. truncatula* after day 30 p.e. In these infected snails, the most differentiated larval forms of *F. hepatica* were free cercariae, rediae containing cercariae, immature larvae, or sporocysts, respectively. The absence of significant differences between frequencies in the 60–80%, 40–59% and 20–39% prevalence classes for each snail group suggested that these four groups of infected snails were always present in natural or experimental infections of *L. truncatula* with *F. hepatica*. As *L. truncatula* is commonly considered to be the principal intermediate host of *F. hepatica* in Europe (Torgerson & Claxton, 1999), the present findings pose the question as to whether the host–parasite relationship existing between the flukes and lymnaeid populations from central France is well balanced, as proposed by Boray (1978) for *L. truncatula*. This is difficult to answer, but an explanation would be to consider that snails dying without cercarial shedding after day 30 p.e. (at 20°C) were not taken into account in the establishment of characteristics for the host–parasite relationship between *L. truncatula* and *F. hepatica*. This suggestion is supported by Boray (1978) for *L. tomentosa*, who reported that 81% of infected snails contained rediae of *F. hepatica* and only 32% of snails shed cercariae. It is also possible that the evolution of the host–parasite relationship between flukes and snail populations in central France is still in progress, despite the presence of fascioliasis in central France for the past two centuries (Taylor, 1965).

In the present study, in four populations of *L. truncatula*, infected snails contained sporocysts with or without immature rediae. According to the classification proposed by Boray (1978) for host–parasite relationships between flukes and lymnaeid snails, these four snail strains must be classed as snails resistant to *F. hepatica* infections, as no cercarial shedding was noted. It would therefore be useful to determine what factors might cause this limited larval development in these snail populations when experimentally infected by this trematode species.

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