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SHORT COMMUNICATION

P. Vignoles · D. Rondelaud · G. Dreyfuss

# A first infection of *Galba truncatula* with *Fasciola hepatica* modifies the prevalence of a subsequent infection and cercarial production in the F1 generation

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**Abstract** Snails from two populations highly susceptible to *Fasciola hepatica* and their F1 generations were subjected to individual bimiracidial exposures to determine if changes noted in infection parameters were due to an effect imposed on the snail by the parasite, or to some other effect such as the food used for the snails. Apart from the higher survival of unexposed parents at day 30 post exposure (p.e.) and their higher shell heights at day 45 p.e., the differences between the survival rates of exposed parents, prevalences of infections, and shell sizes were not significant. In the F1 snails born to previously infected parents, the prevalences of *F. hepatica* infection and cercarial production were significantly lower than those noted for the F1 born to unexposed parents. The survival of these snails and their shell growth did not show any significant variation. The F1 snails born to previously exposed snails would have developed a partial resistance against *F. hepatica* and this process would probably be maximal in the first 2 weeks of larval development inside the snail.

Several factors may influence the success of trematode larval development within the snail. Even though environmental factors affecting the transmission of *Fasciola hepatica* by intermediate hosts have been known a long time (Graczyk and Fried 1999), the effects of biotic factors are not widely understood. The population of *Galba truncatula* (Boray 1978), the snail generation(Rondelaud and Dreyfuss 1997), and the definitive host from which the trematode eggs originated (Rondelaud and Dreyfuss 1995), may influence the success of *F. hepatica* infecting snails, thus inducing variability in the characteristics of snail infections with this trematode under laboratory conditions. The frequency of previous natural encounters between the snail population and the parasite (Rondelaud 1993) was the first explanation proposed to explain this variability. However, this factor might not explain all of the results obtained by our team in experimental infections of snails over the past 10 years. Indeed, low prevalences of snails infected with *F. hepatica* and a small cercarial production were noted in several experiments in which snails originating from populations naturally and highly infected with this trematode were used for experimental infections (unpublished data). In view of these results, it was logical to wonder whether changes in the parameters of snail infections are due to an effect imposed on the snail by the parasite, or to some other effect such as the source of food used to feed the snails. To answer these questions, two experiments were carried out in 2000 and 2002 to determine whether the characteristics of experimental infections with *F. hepatica* in snails collected from natural sites and in their F1 generation born under constant conditions were the same.

The two populations of *G. truncatula* are known to be highly susceptible to *F. hepatica* miracidia of cattle origin, as prevalences of infections higher than 60% were regularly noted in experimentally infected snails. The first population lives in a meadow ditch at Saint Michel de Veisse, department of Creuse (central France). The second inhabits a roadside ditch at Saint Jouvent, department of Haute Vienne. These populations were known to be devoid of any natural trematode infections at the time of experiments as monthly samples of 50 adult snails over the 6 months prior to the experiments revealed no larval forms. The eggs of *F. hepatica* originated from local cattle slaughtered at Limoges. They were collected from the gallbladders of heavily infected animals and incubated for 20 days at 20°C in the dark.

P. Vignoles · D. Rondelaud (✉) · G. Dreyfuss
UPRES EA no. 3174,
Facultés de Pharmacie et de Médecine,
2 rue du Docteur Raymond Marcland,
87025 Limoges, France
E-mail: rondelaud@pharma.unilim.fr
Fax: +33-555-435893

Eight groups of 100 snails each were used in the two experiments. The number of groups made up of parents and F1, the conditions of infection with *F. hepatica*, and the source of food used are listed in Table 1. Two groups of unexposed snails were used as controls, while each snail from the other groups was subjected to a routine bimiracidial exposure at 20°C for 4 h. After exposure, snails were raised for 30 days in polypropylene boxes (1 m×55 cm and 15 cm deep) containing a 2-cm-deep layer of spring water, constantly aerated by bubbling (50 snails per box). Leaves of romaine (Cos) lettuce, used after lying for 5 days in stagnant spring water, were given as food for snails in four groups. In the other four groups, this lettuce was supplemented by a twice-weekly addition of three to four flakes of Tetraphyll (Tetra-Parke-Davis, Courbevoie, France) per box. Water from the original sites was added weekly until day 30 post-exposure (p.e.) to replace that lost by evaporation. The boxes were placed in an air-conditioned room at 20°C under a diurnal photophase of 12 h with 3,000–4,000 lx light intensity.

At day 30 p.e., the surviving snails were individually placed into 35-mm diameter Petri dishes, each containing 2–3 ml of spring water and a piece of lettuce (first experiment), or a piece of lettuce and a small Tetraphyll flake (second experiment). These dishes were maintained in the same air-conditioned room as the breeding boxes. The water was every day changed until snail death. Cercariae were counted and removed from the Petri dishes.

The four parameters studied were: the survival rate of snails in each group at day 30 p.e., the prevalence of *F. hepatica* infections (calculated using the ratio: number of snails shedding cercariae/number of surviving snails at day 30 p.e.), the increase in shell height during the first 45 days of the experiment, and the total number of cercariae shed by each infected snail. A $\chi^2$-test and one-way analysis of variance (Stat-Itcf 1988) were used to determine the levels of significance.

Tables 2 and 3 give the results from the first and second experiments, respectively. Compared to exposed parents, the survival of unexposed parents was significantly greater (Saint Michel de Veisse, $P < 0.05$; Saint Jouvent, $P < 0.05$) at day 30 p.e. A similar finding was also found for the shell heights of unexposed parents at day 45 p.e. (Saint Michel de Veisse, $F = 4.39$, $P < 0.05$; Saint Jouvent, $F = 4.31$, $P < 0.05$). By contrast, the prevalence of infections in parents did not show any significant variation between groups. In the F1 generation, the survival rates of snails at day 30 p.e. and their shell growth at day 45 p.e. did not show any significant variation, whatever the mode of comparison. In the F1 snails born to previously infected parents, the prevalences of *F. hepatica* infections were significantly lower (Saint Michel de Veisse: $P < 0.05$, Saint Jouvent: $P < 0.05$) than those found in the F1 born to unexposed parents, whereas the mean numbers of cercariae were significantly lower (Saint Michel de Veisse: $F = 4.53$, $P < 0.05$; Saint Jouvent: $F = 8.18$, $P < 0.01$).

A comparison of values between the two populations demonstrated that the number of cercariae recorded in the parents from Saint Jouvent was significantly higher ($F = 8.18$, $P < 0.01$). When the F1 generation originated from unexposed parents, the cercarial production of Saint Jouvent snails was also significantly higher ($F = 6.91$, $P < 0.01$), while no significant difference was noted between the values recorded for the F1 snails born to previously infected parents. A comparison of the values for each of the other three parameters did not show any significant differences between the values of exposed parents, those of F1 snails born to unexposed parents, or those of F1 born to previously infected parents.

Compared to parents, the prevalences of *F. hepatica* infections in the F1 generation born to previously exposed parents were decreased by 32.8% in the group of Saint Michel de Veisse and by 38.0% in the Saint Jouvent group, whereas cercarial production was lowered by 56.3% and 66.4%, respectively (Tables 2, 3). These results cannot be explained by the infectivity of the miracidial strain used for snail infection as the decreases were noted in the two experiments, whatever the population of *G. truncatula* and the source of food used to rear the snails. In our opinion, the progeny originating from parents previously infected with *F. hepatica* would have developed a partial resistance against this trematode and this process would probably occur in the first or the first 2 weeks of larval development inside the snail. The lysis of many sporocysts of *F. hepatica* within a few days after miracidial entry, as demonstrated by the presence of tunnelling in the mantle, foot, and other internal organs of *G. truncatula* with abortive infections (Préveraud-Sindou et al. 1994), might explain the lower prevalences noted in these infected descendants. In the

**Table 1** The characteristics of the eight groups of *Galba truncatula* unexposed or exposed to *Fasciola hepatica* miracidia. The F1 generations originated from eggs laid by unexposed or infected parents between day 14 and day 28 of the experiment. These eggs were immediately removed from the parents breeding boxes and placed in other boxes until hatching of newborns and their growth up to 4 mm in height

| Parents, or F1 | Experiment, snail population (and source of food) | |
|---|---|---|
| | First experiment, Saint Michel de Veisse, (romaine lettuce) | Second experiment, Saint Jouvent (mixed diet) |
| Parents | Unexposed snails | Unexposed snails |
| | Exposed snails | Exposed snails |
| F1 born from unexposed parents | Exposed snails | Exposed snails |
| F1 born from infected parents | Exposed snails | Exposed snails |

**Table 2** Characteristics of *Fasciola hepatica* infections in the parents and F1 generation from the population of Saint Michel de Veisse (first experiment)

| Parameters | Parents | | F1 generation from | |
|---|---|---|---|---|
| | Unexposed snails | Exposed snails | Unexposed parents | Infected parents |
| Survival (%) of snails at day 30 p.e. | 98.0 | 75.0 | 78.0 | 68.0 |
| Number of snails shedding cercariae (and prevalence of infection in %) | 0 | 61 (81.3) | 66 (84.6) | 33 (48.5) |
| Mean value and (SD) for the shell height of snails (mm) at day 45 p.e. | 7.5 (0.4) | 6.9 (0.4) | 7.0 (0.5) | 6.4 (0.8) |
| Mean value and (SD) for the number of cercariae | 0 | 142.5 (75.8) | 155.3 (75.9) | 62.3 (41.2) |

**Table 3** Characteristics of *Fasciola hepatica* infections in the parents and F1 generation from the population of Saint Jouvent (second experiment)

| Parameters | Parents | | F1 generation from | |
|---|---|---|---|---|
| | Unexposed snails | Exposed snails | Unexposed parents | Infected parents |
| Survival (%) of snails at day 30 p.e. | 97.0 | 71.0 | 72.0 | 71.0 |
| Number of snails shedding cercariae (and prevalence of infection in %) | 0 | 56 (78.8) | 63 (87.5) | 29 (40.8) |
| Mean value and (SD) for the shell height of snails (mm) at day 45 p.e. | 7.8 (0.5) | 7.0 (0.6) | 7.2 (0.7) | 6.8 (0.7) |
| Mean value and (SD) for the number of cercariae | 0 | 322.3 (114.6) | 345.6 (138.9) | 108.3 (101.5) |

same way, the fewer cercariae might be the result of a low redial burden, probably limited to several mother rediae produced by each surviving sporocyst and a few daughter rediae. An argument in support of this interpretation is the limited redial burden of *F. hepatica* which developed in snails exposed to miracidia after experimental desiccation and activation in water (Rondelaud 1994), or after poisoning by sub-lethal doses of a molluscicide (Rondelaud 1995).

The mechanism responsible for the lower performances in F1 snails born to previously-exposed snails is more difficult to explain, as these characteristics were observed in 4-mm high *G. truncatula* and, consequently, in 4-week-old snails (1 mm of growth per week for this species at 20°C, Gold 1980) at the date of miracidial exposure. If the time necessary for egg incubation (a mean of 10 days at 20°C, Morel-Vareille 1973) is added to the duration of snail lifespan, the lower performances noted in these snails would occur 6 weeks or more after egg laying. The breeding conditions which were present in the parent boxes could not come into question as the eggs laid by the parents were removed just after they were deposited (Table 1). As these lower performances were observed only in the F1 generation born to previously exposed parents, the most likely hypothesis would be that transmission of one or several factors from infected parents to their progeny via the eggs occurs. One of these factors might be present in the haemolymph of the infected parents, such as schistosomin identified in *Lymnaea stagnalis* infected with *Trichobilharzia ocellata* (Jong-Brink 1995), or different glycoconjugate antigens which were found in snails infected with *Schistosoma mansoni* or *Schistosoma haematobium* (Schmitt et al. 2002). Further studies are necessary to verify these last hypotheses.

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