Journal of Helminthology (2003) **77**, 275–277 DOI: 10.1079/JOH2003177

Research Note

The experimental production of *Fasciola hepatica* metacercariae from three aquatic populations of *Galba truncatula*

P. Vignoles[1], L. Favennec[2], D. Rondelaud[1]* and G. Dreyfuss[1]

[1]UPRES-EA no. 3174, Facultés de Pharmacie et de Médecine, 2 rue du Docteur Marcland, 87025 Limoges Cedex, France:
[2]UPRES-EA no. 3234, Faculté de Médecine-Pharmacie, 22 boulevard Gambetta, 76183 Rouen Cedex, France

Abstract

Laboratory investigations on three aquatic populations of *Galba truncatula*, originating from the Peruvian Altiplano and French Massif Central, were carried out during three successive snail generations to determine if these populations might be successfully used for the metacercarial production of *Fasciola hepatica* under experimental conditions. High numbers of surviving snails at day 30 post-exposure (>70%), high prevalences of *F. hepatica* infections (>60%), and prolonged productions of cercariae for a mean period of 35 to 47 days were observed in the three populations, whatever the snail generation. In the Peruvian population, metacercariae of *F. hepatica* significantly decreased in numbers from a mean of 251 in the parent snails to 124 per snail in the F_2 generation, whereas no significant variation was observed in the two French populations. As these aquatic snails rarely emerged out of water, the use of these populations for the commercial production of *F. hepatica* metacercariae was of great interest, because the daily time spent watching the breeding boxes of snails was clearly shorter, thereby reducing the cost of producing metacercariae compared with using amphibious snails reared with romaine lettuce.

The snail *Galba truncatula* (= *Lymnaea truncatula*) acts as an intermediate host in the life cycle of the liver fluke *Fasciola hepatica*. As fascioliosis caused by this parasite is an economically important disease of livestock, research laboratories regularly request metacercariae of *F. hepatica* for the purpose of infecting ruminants under experimental conditions and to study the development of flukes, or to determine the efficiency of new anthelmintics against them. In order to obtain a ready supply of metacercariae, it is necessary to maintain experimentally-infected snails under laboratory conditions. Some methods and several sources of food have already been proposed by different authors for the rearing of *G. truncatula* (Kendall, 1949; Boray, 1969; Osborn *et al.*, 1982; Rondelaud *et al.*, 2002).

Despite these reports, the breeding of *G. truncatula* in the laboratory is difficult, as the snail is known to be amphibious and suitable conditions must be provided for its growth (Boray, 1969). Indeed, as in all Lymnaeidae, *G. truncatula* finds its optimal habitat in perennial aquatic sites, but in contrast to its congeneric species, it can thrive very well on mud flats in the absence of free water, by using supracapillary water from the soil (Moens, 1991). However, all populations of *G. truncatula* do not have the same requirements for moisture. Most European populations of this snail are markedly amphibious (Taylor, 1965), whereas those originating from many French highland habitats (Rondelaud & Mage, 1992) or the South

*Author for correspondence
Fax: 33 555 435893
E-mail: rondelaud@pharma.unilim.fr

American Altiplano (Mas-Coma *et al.*, 1999) are more aquatic and are rarely encountered in mud, out of water. In view of these findings, the following two questions arose: (i) can the more aquatic populations of *G. truncatula* be successfully used for the production of digenean metacercariae? and (ii) do the characteristics of *Fasciola* infections change when several subsequent generations of snails are reared under laboratory conditions? To answer these questions, experiments were carried out using three populations of *G. truncatula* to analyse the characteristics of experimental infections with *F. hepatica* and also to monitor any changes in their susceptibility to infections when their F_1 and F_2 generations were subsequently subjected to miracidial exposures and reared under constant conditions.

The first population of *G. truncatula* originated from the Peruvian Altiplano (district of Cajamarca), whereas the two other populations were from central France, near Aubusson (department of Creuse). These three populations were known to be frequently encountered in water, whatever the season (Rondelaud & Mage, 1992; Mas-Coma *et al.*, 1999). One hundred snails, measuring 4 mm in height and belonging to the spring generation, were collected from each habitat and were progressively acclimatized for a 48-h period to a constant temperature of 20°C before being exposed to miracidia. Eggs of *F. hepatica* were collected from the gallbladders of heavily infected cattle at the slaughterhouse of Limoges and were incubated in complete darkness for 20 days at 20°C.

Bimiracidial infections of 4-mm high snails (100 per population and per snail generation) were performed for the parents from each snail population, the F_1, and the F_2 generations. Every year, the three groups of snails (one per population of *G. truncatula*) were infected with the same miracidial isolate. Snails from the nine groups were subsequently raised for 30 days in polypropylene boxes, 1 m by 55 cm and 15 cm high (50 snails per box). Each box contained small stones and a 2-cm deep layer of water, constantly aerated. Snails were fed with steeped romaine lettuce. These boxes were placed under constant temperature conditions of 20°C and a diurnal photophase of 3000–4000 lux light intensity. At day 30 post-exposure (p.e.), each surviving snail was placed in a 35-mm diameter Petri dish, containing 2–3 ml of spring water and a piece of lettuce, and the dishes maintained at 20°C. Each day, metacercarial counts were performed and the water in the dishes was changed until the death of the snails.

The parameters studied were the survival rates of snails in each group at day 30 p.e., the prevalence of *F. hepatica* infection (calculated using the ratio between the number of cercariae-shedding snails and that of surviving snails at day 30 p.e.), the life span of infected snails between exposure and their death after cercarial shedding, the duration of cercarial shedding, and the total number of metacercariae for each infected snail. A comparison test of experimental frequencies and a one-way analysis of variance (Stat-Itcf, 1988) were used to establish levels of significance.

Table 1 gives the principal characteristics of these experimental procedures. Firstly, high numbers of surviving snails at day 30 p.e. (>70%) and high prevalences of *F. hepatica* infections (>60%) were found in the nine snail groups. A comparison of the percentages recorded for each of the parameters showed no significant differences. Secondly, the long life-span of cercariae-shedding snails (more than 87 days) and the prolonged production of cercariae (a mean of 35–47 days for the shedding period) were noted in all groups, whatever the snail generation. There were no significant differences between the mean values of each parameter. Lastly, the mean number of *F. hepatica* metacercariae in the Peruvian snails significantly decreased ($F = 3.51$, $P < 0.005$) from 251 in the parents to 124 in the F_2 generation of snails, whereas no significant variation was observed in the two French populations.

Table 1. Characteristics of experimental infections of three populations of *Galba truncatula* with *Fasciola hepatica* in parent snails and the F_1 and F_2 generations.

Parameters	Generation	Population of *Galba truncatula*		
		Peruvian Altiplano	France 1*	France 2*
Survival rate (%) at day 30 p.e.	Parents	76.0	71.0	74.0
	F_1	77.0	71.0	73.0
	F_2	71.0	73.0	76.0
No. of cercariae-shedding snails (and prevalence of infection in %)	Parents	54 (71.0)	47 (68.2)	49 (66.2)
	F_1	49 (63.6)	47 (66.1)	45 (61.6)
	F_2	51 (71.8)	50 (68.4)	52 (68.4)
Life span of cercariae-shedding snails (days): mean ± SD	Parents	91.1 ± 13.3	98.3 ± 15.2	90.2 ± 10.7
	F_1	90.5 ± 11.2	94.2 ± 13.1	92.7 ± 12.5
	F_2	87.5 ± 9.3	91.4 ± 15.4	95.0 ± 9.1
Duration of cercarial shedding (days): mean ± SD	Parents	38.5 ± 17.5	47.2 ± 21.7	35.3 ± 18.1
	F_1	41.2 ± 14.3	43.7 ± 19.7	39.9 ± 14.0
	F_2	35.6 ± 10.2	42.8 ± 17.4	37.2 ± 16.5
No. of metacercariae: mean ± SD	Parents	251.5 ± 106.3	94.0 ± 71.2	116.5 ± 89.0
	F_1	177.3 ± 95.5	119.2 ± 95.3	147.0 ± 108.7
	F_2	124.1 ± 78.7	103.1 ± 89.2	123.4 ± 91.2

*France 1 (Masvaudier, commune of Saint Michel de Veisse, department of Creuse); France 2 (near the village of Vallières, department of Creuse).

https://doi.org/10.1079/JOH2003177 Published online by Cambridge University Press

Apart from the total number of metacercariae in Peruvian snails, the characteristics of infections in the three populations of aquatic snails were the same, whatever the snail generation. These results agree with the findings of Rondelaud & Dreyfuss (1997) and Abrous *et al.* (1998), for example, in amphibious populations of *G. truncatula* subjected to bimiracidial infections with *F. hepatica* and subsequently reared on romaine lettuce. However, as these aquatic snails rarely emerged out of water and, consequently, did not withdraw inside their shell for fasting when moisture disappeared from immersed areas, the use of these *G. truncatula* populations for commercial production of *F. hepatica* metacercariae was of great interest, because the time spent observing the breeding boxes of snails on a daily basis was much reduced and the cost price of 100 *F. hepatica* metacercariae produced by this method was lower (13.6 US $ compared with 15.7 $ using amphibious snails reared with romaine lettuce: Rondelaud *et al.*, 2002).

The high number of *F. hepatica* metacercariae in the parent generation of Peruvian snails may be related to the increased infectivity of miracidia when an allopatric strain of parasite was used for experimental infections of snails (Gasnier *et al.*, 2000). According to Gasnier *et al.* (2000), the Spanish flukes were more efficient for French *G. truncatula* than for Spanish snails. Conversely, the steady decrease in metacercarial production noted in the F_1 and F_2 snails of the Peruvian population is more difficult to interpret. It is possible that the higher efficiency of allopatric miracidia in snail infections would occur only in the parent generation of *G. truncatula* and would decrease in the subsequent generations. If this hypothesis is valid, this numerical decrease in metacercarial production in the F_1 and F_2 generations of the Peruvian population would be a consequence of progressive adaptations of these *G. truncatula* to an allopatric isolate of miracidia. An argument in support of this last assumption is shown by the number of *F. hepatica* metacercariae noted in the sympatric combinations of French snails and miracidia, whatever the snail generation.

References

Abrous, M., Roumieux, L., Dreyfuss, G., Rondelaud, D. & Mage, C. (1998) Proposition d'une technique simple pour la production métacercarienne de *Fasciola hepatica* Linné à partir du mollusque *Lymnaea truncatula* Müller. *Revue de Médecine Vétérinaire* **149**, 943–948.

Boray, J.C. (1969) Experimental fascioliasis in Australia. *Advances in Parasitology* **7**, 95–210.

Gasnier, N., Rondelaud, D., Abrous, M., Carreras, F., Boulard, C., Diez-Banos, P. & Cabaret, J. (2000) Allopatric combination of *Fasciola hepatica* and *Lymnaea truncatula* is more efficient than sympatric ones. *International Journal for Parasitology* **30**, 573–578.

Kendall, S.B. (1949) Nutritional factors affecting the rate of development of *Fasciola hepatica* in *Limnaea truncatula*. *Journal of Helminthology* **23**, 179–190.

Mas-Coma, S., Anglés, R., Esteban, J.G., Bargues, M.D., Buchon, P., Franken, M. & Strauss, W. (1999) The northern Bolivian Altiplano: a region highly endemic for human fascioliasis. *Tropical Medicine and International Health* **4**, 454–467.

Moens, R. (1991) Factors affecting *Lymnaea truncatula* populations and related control measures. *Journal of Medical and Applied Malacology* **3**, 73–84.

Osborn, G.D., Gron, N. & Simmons, D. (1982) Maintenance and infection of the mud snail *Lymnaea truncatula* for *Fasciola hepatica* studies. *Journal of the Institute of Animal Technicians* **33**, 1–5.

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.

Rondelaud, D. & Mage, C. (1992) *Lymnaea truncatula* Müller: les conséquences d'une seule génération annuelle sur les caractéristiques de l'infestation par *Fasciola hepatica* L. *Revue de Médecine Vétérinaire* **143**, 843–946.

Rondelaud, D., Abrous, M. & Dreyfuss, G. (2002) The influence of different food sources on cercarial production in *Lymnaea truncatula* experimentally infected with Digenea. *Veterinary Research* **33**, 95–100.

Stat-Itcf (1988) *Manuel d'utilisation*. 210 pp. Institut technique des céréales et des fourrages, Service des études statistiques, Boigneville, France.

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

(Accepted 24 February 2003)