Parasitol Res (2012) 111:2011–2016
DOI 10.1007/s00436-012-3049-9

ORIGINAL PAPER

Intermediate snail hosts of French *Fasciola hepatica*: *Lymnaea neotropica* and *Lymnaea viatrix* are better hosts than local *Galba truncatula*

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Received: 22 May 2012 / Accepted: 13 July 2012 / Published online: 4 August 2012

Abstract Allopatric and sympatric infections of *Lymnaea neotropica* and *Lymnaea viatrix* var. *ventricosa* with Argentinean and French isolates of *Fasciola hepatica* were carried out to determine the capacity of these snails to produce metacercariae and to verify if this capacity changed with snail generation. The same process was also made with a French population of *Galba truncatula* known to be highly susceptible to French isolates of the parasite. In each lymnaeid species separately considered, the survival rate at day 30 post-exposure and prevalence of *F. hepatica* infection in the group infected with Argentinean miracidia were significantly greater than those recorded in the corresponding French one. Compared to infected *G. truncatula*, both South American lymnaeids had longer patent periods and produced a higher number of metacercariae. The highest infections were noted with *L. v. ventricosa*. In the three snail species, metacercarial production was more important with the Argentinean isolate of miracidia than with the French one. If three successive generations of *L. v. ventricosa* are exposed to the same French isolate of miracidia, cercarial production significantly increased from parents to the F2 generation, while the other characteristics of infection only showed insignificant variations. *L. neotropica* and *L. v. ventricosa* are better intermediate hosts for French *F. hepatica* than local *G. truncatula*. The numerical increase of shed cercariae in the F1 and F2 generations of *L. v. ventricosa* demonstrates a rapid adaptation of this species to the French isolate of the parasite.

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Introduction

According to the theory of local adaptation in parasite systems, a digenean should perform better on its local (sympatric) snail hosts than on foreign (allopatric) hosts and thus would become adapted to these local hosts (Lively and Dybdahl 2000; Greischar and Koskella 2007; King et al. 2011). The efficiency of such sympatric combinations has been shown in several snail-parasite systems (Muñoz-Antoli et al. 2010; Mostafa and el-Dafrawy 2011). However, this theory was not verified in other snail-parasite systems (Prugnolle et al. 2006; Osnas and Lively 2011). This variable nature of results was also found in the *Galba truncatula–Fasciola hepatica* system. Local adaptation between both partners was verified in the field (Abrous et al. 1999, 2000) and even during the larval development of *F. hepatica* within its snail host (Belfaiza et al. 2005). However, the better fitness noted in these sympatric infections cannot be generalised because allopatric combinations of *G. truncatula* and *F. hepatica* are sometimes more efficient for cercarial production than sympatric ones. Spanish isolates of *F. hepatica* miracidia, coming from cattle infections, were more infective to French populations of *G. truncatula* than miracidial isolates originating from central France (Gasnier et al. 2000). A higher prevalence of *F. hepatica* infection and a greater cercarial production were also noted in allopatric combinations of

French snails and Moroccan miracidia (Goumghar et al. 2001). In the report by Coelho et al. (2009), the results were more conflicting. Allopatric combinations between *Lymnaea columella* and *F. hepatica* miracidia were more efficient than sympatric ones when snails living in south-western Brazil (Itajubá) were used, but the opposite was observed in allopatric infections with snails from south-eastern Brazil and also in both types of sympatric infections. According to Coelho et al. (2009), the host–parasite relationships may vary with the geographical origin of snails and that of flukes involved.

In view of these findings, one may wonder if the better results noted by Gasnier et al. (2000) and Goumghar et al. (2001) during allopatric infections of French snails with Spanish and Moroccan isolates of *F. hepatica* be found when lymnaeid species other than *G. truncatula* are used for experimental infections. To answer this question, it was necessary to select lymnaeids known to sustain larval development of *F. hepatica* and to use the experimental protocol already applied by the above-mentioned authors because the characteristics of snails and parasites and also the experimental protocol used by biologists were important for detecting local adaptation in coevolving species interactions (Hoeksema and Forde 2008). For this reason, we have chosen two populations of South American lymnaeids: *Lymnaea neotropica* and *L. viatrix* var. *ventricosa*, which are currently raised under laboratory conditions at CEDIVE (Faculty of Veterinary Sciences, National University of La Plata, Buenos Aires, Argentina). Both species belong to the *Galba* group (amphibious snails) and can be identified by the relative length of their penis sheath and preputium (Pointier et al. 2006) and molecular biology (Bargues et al. 2007). Their biological differences are still misunderstood because these snail species were confused under the name of *Lymnaea viatrix* before the 2000s and were only separated from each other since 2007 (Bargues et al. 2007). Allopatric and sympatric infections of these snail populations with Argentinean and French isolates of *F. hepatica* were thus carried out to compare their potential for metacercarial production and to verify if this capacity can be noted in further generations of these snails when the method by Rondelaud et al. (2007) for the breeding of lymnaeids was used. Controls were constituted by a French population of *G. truncatula*, infected and raised according to the same protocol.

Materials and methods

Snails and *F. hepatica* isolates

The two South American lymnaeids were raised in CEDIVE since 2008 (*L. neotropica*) and 1996 (*L. v. ventricosa*). The first species came from a population living in San Pedro, Buenos Aires, Argentina (33° 40′ S, 59° 39′ W), while *L. v. ventricosa* originated from Paysandú, Uruguay (36° 00′ S, 57° 30′ W). As the systematics of South American lymnaeids is controversial and cannot be made with the single use of morphological criteria (Duffy et al. 2009; Mera y Sierra et al. 2009), the identification of these two species was performed using PCR-RFLP and sequencing of ITS-1 of their nuclear rDNA (Sanabria et al. 2012). A French population of *G. truncatula* was used as controls and was living in a road ditch at Chézeau Chrétien, commune of Chitray, department of Indre, central France (46° 40′ 27″ N, 1° 21′21″ E). This last population was known to be highly susceptible (>60 %) to French miracidia of *F. hepatica* (Dreyfuss et al. 2007). Four-millimetre-high snails (Table 1) were collected from each population. Eggs of the Argentinean isolate of *F. hepatica* were collected in the gall bladders of cattle from Buenos Aires, while those of the French isolate came from the gall bladders of cattle at the slaughterhouse of Limoges (France). All the eggs were washed several times with spring water and were incubated for 12 days at 24 °C in the dark (Ollerenshaw 1971).

Table 1 Main characteristics of snail populations and miracidial isolates used in the two experiments

Experiment (purpose)	Snail population (origin)	Origin of *F. hepatica*	Total number of preadult snails at exposure
A (aptitude of each snail population as an intermediate host of *F. hepatica*)	*L. neotropica* (Argentina)	Argentina	100
		France	100
	L. v. ventricosa (Uruguay)	Argentina	100
		France	100
	G. truncatula (France)	Argentina	100
		France	100
B (development of *F. hepatica* infection in *L. v. ventricosa* from parents to the F2 generation)	Parents	France	50
	F1		50
	F2		50

Experimental infections of snails

Table 1 gives the purpose of each experiment, the snail strain, the origin of *F. hepatica* miracidia and the total number of preadult snails (shell height, 4 mm) in each snail group. In experiment (A), the three populations of snails were subjected to Argentinean or French miracidia of *F. hepatica*. In contrast, only *L. v. ventricosa* was used in experiment (B) because these snails were the most efficient for cercarial production with the French isolate of *F. hepatica* miracidia. The F1 snails originated from eggs laid by their infected parents between weeks 2 and 5 post-exposure (p.e.). A similar protocol was used for the F2 generation. This protocol for the F1 and F2 generations was chosen in order that these descendants have a first contact with the parasite through their infected parents.

A bimiracidial exposure was performed for each snail. The lymnaeids were then raised per groups of ten individuals in 14-cm Petri dishes for 30 days according to the method by Rondelaud et al. (2007). Food constituted of dried leaves of lettuce and dead leaves of grass (*Molinia caerulea*), while several stems of spring moss (*Fontinalis* sp.) ensured oxygenation of the water layer (concentration of dissolved calcium in spring water, 35 mg/l). Water and food, if necessary, were changed every day. The Petri dishes were placed in an air-conditioned room under the following conditions: a constant temperature of 20 °C (±1 °C), natural photoperiod of 10-h light. At day 30 p.e., each surviving snail was individually placed in a 35-mm Petri dish with pieces of dead grass, lettuce and spring moss. These last recipients were also kept at 20 °C. A daily surveillance was performed to change water and food, if necessary. If metacercariae were present, they were counted and removed from the Petri dish. This surveillance was applied up to the death of each snail.

Parameters studied

The first two parameters were snail survival at day 30 p.e. and the prevalence of *F. hepatica* infection (calculated in relation to the number of surviving snails at day 30 p.e.). For each population, the difference between the values recorded in the Argentinean and the French groups was analysed using a χ^2 test. The growth of cercariae-shedding (CS) snails during the experiment, the length of the prepatent period, the length of the patent period and the total number of metacercariae were also taken into account. Individual values recorded for these last five parameters were averaged, and their standard deviations were calculated, taking into account snail groups. One-, two- or three-way analysis of variance was used to establish levels of significance. All the statistical analyses were made using the Statview 5.0 software.

Results

Lymnaeid species and snail infection

Compared to French *G. truncatula* infected with Argentinean miracidia (Table 2), snail survival at day 30 p.e. was significantly higher (χ^2=7.29, P<0.05) in both South American species. In contrast, in groups with French miracidia, survival was significantly lower (χ^2=12.15, P<0.01) for *L. v. ventricosa* than for the other two lymnaeids. In each species separately considered, the survival rate in the group infected with Argentinean miracidia was significantly greater (*G. truncatula*, χ^2=5.21, P<0.05; *L. neotropica*, χ^2=37.7, P<0.001; *L. v. ventricosa*, χ^2=11.07, P<0.001). The prevalence of *F. hepatica* infection in the three lymnaeids did not significantly differ from each other, whatever the origin of miracidia; whereas that noted in the Argentinean group of each snail species was significantly greater (*G. truncatula*, χ^2=23.73, P<0.001; *L. neotropica*, χ^2=30.6, P<0.001; *L. v. ventricosa*, χ^2=14.66, P<0.001) than that recorded in the corresponding French one. The growth of CS *L. neotropica* and *L. v. ventricosa* during the experiment was significantly (F=87.19, P< 0.001) greater than that of *G. truncatula*, whereas the miracidial origin had no clear effect on this parameter.

The lengths of prepatent periods (Table 3) ranged in the same scale of values, and no significant difference was

Table 2 Survival of exposed snails at day 30 post-exposure, prevalence of infection and growth of CS snails in the three species of lymnaeids infected with Argentinean or French miracidia (experiment A)

Snail population	Origin of *F. hepatica*	Number of surviving snails at day 30 p.e. (%)	Number of CS snails (prevalence in %)	Mean growth (SD) of CS snails during the experiment (mm)
G. truncatula	Argentina	76 (76.0)	23 (30.2)	2.0 (0.2)
	France	61 (61.0)	44 (72.1)	2.0 (0.1)
L. neotropica	Argentina	89 (89.0)	18 (20.6)	2.8 (0.4)
	France	70 (70.0)	32 (69.5)	2.8 (0.4)
L. v. ventricosa	Argentina	87 (87.0)	29 (32.5)	3.1 (0.3)
	France	46 (46.0)	44 (62.8)	2.9 (0.4)

Table 3 Prepatent period, patent period and total number of metacercariae noted in the three species of lymnaeids infected with Argentinean or French miracidia (experiment A)

Snail population	Origin of *F. hepatica*	Mean length (SD) in days		Mean number (SD) of metacercariae	
		Prepatent period	Patent period	Per CS snail	Per snail exposed to miracidia
G. truncatula	Argentina	38.4 (1.7)	85.9 (22.4)	897.3 (229.9)	206.3
	France	39.3 (2.0)	18.2 (8.2)	222.1 (93.1)	97.7
L. neotropica	Argentina	42.9 (2.9)	65.0 (33.2)	670.1 (375.9)	120.6
	France	43.0 (3.0)	51.1 (17.9)	404.6 (224.4)	129.4
L. v. ventricosa	Argentina	42.3 (1.5)	96.3 (34.6)	1383.0 (698.6)	401.0
	France	42.0 (1.9)	54.0 (27.5)	542.3 (420.4)	238.6

CS cercariae-shedding snail

noted. In the case of patent periods, higher values were found in the three Argentinean groups, thus indicating the significant effect of miracidial origin (F=30.37, P<0.001). The shell growth of CS snails had also a significant influence (F=137.62, P<0.001), whereas that of lymnaeid species was insignificant. Similar findings were also noted for metacercariae, with higher numbers in the Argentinean groups. The highest values were noted for *L. v. ventricosa*, with a mean of 1,383 metacercariae in the Argentinean group and 542 in the French one. Significant effects were noted for lymnaeid species (F=15.04, P<0.001), the origin of miracidia (F=26.51, P<0.001) and snail growth (F=134.44, P<0.001).

If the metacercarial production of each group is related to the number of snails at miracidial exposure (Table 3), the highest difference between the values of allopatric and sympatric groups was noted for *L. v. ventricosa*, while the numbers for *L. neotropica* were close to each other.

Generation of *L. v. ventricosa* and snail infection with French miracidia

Table 4 gives the values of the six parameters. Even if there was a slight increase in the survival rates at day 30 p.e. from parents to the F2 generation, this variation was insignificant. Similar findings were also noted for the prevalence of *F. hepatica* infections, the shell growth of CS snails during experiment, the length of the prepatent period and that of the patent period. In contrast, the number of metacercariae significantly increased in time, going from a mean of 364.7 cercariae in parents to 565.1 in the F2 group.

Discussion

Among the three lymnaeid species experienced in the present study, *L. v. ventricosa* was the most productive with a mean of 1,383 metacercariae in the Argentinean group and 542 in the French one. Moreover, these cercariae were shed during a long patent period of 96.3 and 54.0 days, respectively. Such values were never obtained with French populations of *G. truncatula* when they were experimentally infected with sympatric isolates of *F. hepatica* miracidia (Rondelaud et al. 2004, 2007). The differences noted between values of these two parameters can be partly explained by the report of Mas-Coma et al. (2001). When two populations of *Lymnaea sp*. (unidentified species), coming from Northern Altiplano (Bolivia), were infected with sympatric miracidia of *F. hepatica*, the authors noted a longer cercarial shedding period (up to 85 days at 20 °C) and a higher cercarial production (up to 589 metacercariae). According to Mas-Coma et al. (2001), these findings may be interpreted as the consequence of strategies associated with adaptation to high altitude conditions. Even if South American lymnaeids would be better intermediate hosts for local

Table 4 Main characteristics of *F. hepatica* infection in three groups of *L. v. ventricosa* according to snail generation (experiment B)

Parameters	Parents	F1	F2
Number of surviving snails at day 30 p.e. (%)	34 (68.0)	35 (70.0)	38 (76.0)
Number of CS snails (prevalence %)	22 (64.7)	25 (71.4)	31 (81.5)
Mean growth (SD) of CS snails during the experiment (mm)	2.7 (0.3)	2.7 (0.3)	2.8 (0.3)
Mean length (SD) of the prepatent period (days)	41.9 (2.1)	42.0 (1.7)	42.1 (2.0)
Mean length (SD) of the patent period (days)	48.0 (17.3)	57.8 (24.9)	59.6 (28.6)
Mean number (SD) of metacercariae	364.7 (192.4)	462.3 (201.1)	565.1 (313.4)*

CS cercariae-shedding snails
*P<0.05 (Anova, F=6.62)

F. hepatica, this first interpretation did not explain all findings noted in the present study, and another complementary explanation must be proposed. In our opinion, the values recorded for the length of patent period and metacercarial production between Argentinean and French groups of snails, whatever lymnaeid species, demonstrated that the Argentinean isolate of *F. hepatica* used in this experiment seemed little aggressive contrary to the French isolate of the parasite. This last result might be the consequence of anthelmintics used by farmers to treat infected cattle (according to Reynal (2001), most farmers in central France used triclabendazole to treat cattle against fasciolosis) and increased infectivity of local miracidia towards populations of *G. truncatula* (Dreyfuss et al. 2007).

Even when the numerical variations noted for five parameters in parent *L. v. ventricosa* and their descendants were insignificant, snail survival, prevalence of infection, the patent period and the number of metacercariae showed slight increases from parents to the F2 generation. These results suggested a rapid adaptation of *L. v. ventricosa* to the French isolate of the parasite. Similar adaptations of lymnaeids to an unusual isolate of *F. hepatica* over several generations of the snail were already reported for other snail-parasite systems such as the *Lymnaea peregra–F. hepatica* model (Boray 1966, 1969) or that concerning *Omphiscola glabra* and *Paramphistomum daubneyi* in central France (Dreyfuss et al. 2010), for example. This finding demonstrates the capacity of the South American *L. v. ventricosa* as an intermediate host for another isolate of *F. hepatica*, even if cattle-derived miracidia from central France showed an increased infectivity towards snails from several years (Dreyfuss et al. 2007).

In conclusion, even if the present study demonstrates that allopatric *L. neotropica* and *L. v. ventricosa* are better intermediate hosts for French miracidia of *F. hepatica*, sympatric infections of South American lymnaeids with Argentinean miracidia were the most productive for metacercariae. This last finding throws the role of allopatric snails for parasite production into question. In our opinion, the above-mentioned results can only be explained by the report of Coelho et al. (2009). According to these authors, the success of a snail infection for the production of cercariae depended on the geographic origin of snails and flukes used for experiments.

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