Paramphistomum daubneyi and *Fasciola hepatica*: the prevalence of natural or experimental infections in four species of freshwater snails in eastern France

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Abstract

Parasitological investigations were performed in July and September–October 1997 in six farms located in the department of Saône et Loire (eastern France) to determine the prevalence of natural infections with *Paramphistomum daubneyi* and *Fasciola hepatica* in four species of freshwater snails. Cercaria-containing rediae of *P. daubneyi* and/or *F. hepatica* were found in *Lymnaea palustris* (one snail only) and *Lymnaea truncatula*. Some living sporocysts and immature rediae were noted in *Lymnaea ovata* (*P. daubneyi* or *F. hepatica*) and in *Physa acuta* (*P. daubneyi* only). The prevalence of each trematode infection was often less than 10%. Experimental infections of juvenile and preadult snails (1 and 4 mm in height, respectively) were also performed to test the susceptibility of these four snail species to *P. daubneyi*, either singly or in combination with *F. hepatica*. Both 1 and 4 mm high *L. truncatula* could sustain the full development of *P. daubneyi*, whether in single or double infections. In *L. palustris* dually exposed to both trematodes, cercaria-containing rediae of *P. daubneyi* were found in one juvenile and one preadult snails, while immature infections were noted in ten juvenile and two preadult snails. The overall prevalence of *P. daubneyi* infection in *L. palustris* was 11.1% in juvenile snails and 2.1% in preadults. Larval forms of *P. daubneyi* and *F. hepatica* were only noted in dually-exposed juvenile *L. ovata* and *P. acuta*. In *L. ovata*, mature and immature rediae of *F. hepatica* were detected in 17.6% of snails, while immature rediae of *P. daubneyi* were noted in 4.4% of snails. In *P. acuta*, only immature infections were detected (5.1% of snails with *P. daubneyi*, and 1.2% with *F. hepatica*). These results demonstrated that *Lymnaea* species other than *L. truncatula* could sustain the full development of *P. daubneyi* and that immature larvae of this trematode might be found in naturally- or experimentally-infected *L. ovata* and *P. acuta*.

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Introduction

In western Europe, some *Lymnaea* species act as intermediate hosts in the life cycle of *Paramphistomum daubneyi* and *Fasciola hepatica. Lymnaea truncatula* (Dinnik, 1962) and *L. peregra* (Sey, 1979) have been reported for the former species. In contrast, the list of snail species is clearly wider for *F. hepatica*. If *L. truncatula* is mentioned by numerous workers as the principal host snail (Euzeby, 1971; Boray, 1978), the other French *Lymnaea*, with the exception of *Lymnaea auricularia*, may sustain the larval development of *F. hepatica* on condition that miracidial exposure occurs in the early days in the life of the snail (Busson *et al*., 1982; Vareille-Morel *et al*., 1994).

The question of the intermediate hosts of *P. daubneyi* is more complex, as larval forms of *P. daubneyi* and those of *F. hepatica* may develop in the same snail. This process was noted in *L. truncatula* (Chipev *et al*., 1985; Augot *et al*., 1996). It was also reported in *Lymnaea glabra*: single infections of this species with *P. daubneyi* were negative, whereas snails dually infected with *P. daubneyi* and *F. hepatica* shed cercariae of both trematodes (Abrous *et al*., 1996). As *L. truncatula* was not the only freshwater species in meadows and was sometimes lacking (Abrous *et al*., 1998), the following questions arose: might pulmonate species other than *L. truncatula* act as natural intermediate hosts in the life cycle of *P. daubneyi* ? Was the full development of *P. daubneyi* in these snails the consequence of single infections with this trematode, or from double infections with the combination *P. daubneyi* + *F. hepatica* ? To answer the first question parasitological investigations in four species of freshwater snails were performed in 1997 in six farms located in the department of Saône et Loire (eastern France) and known to have a history of paramphistomosis in their cattle. To answer the second question we have tested the susceptibility of these four snail species to *P. daubneyi*, either singly or in combination with *F. hepatica*.

Materials and methods

Field investigations

The six cattle-rearing farms were located in the department of Saône et Loire, and table 1 gives their principal characteristics. Cases of paramphistomosis in cattle were detected periodically in all these farms (Degueurce, 1998). Three snail species were recovered in five farms: *L. truncatula* (ten habitats), *L. ovata* (three habitats), and *Physa acuta* (two habitats). *Lymnaea palustris* (one habitat) and *P. acuta* (one habitat) were found in the last farm.

Snail sampling was performed in different habitats located in the six farms July and September–October 1997, as most cercarial sheddings from naturally-infected snails occurred during these months in central France. Only snails measuring 3 mm or more in height were searched visually and collected. They were dissected under the stereomicroscope to detect larval forms of trematodes and to classify infected snails into four groups: snails infected with *F. hepatica* only, *P. daubneyi* only, or both; snails infected by other trematode species (see table 2). The criteria permitting the identification of *P. daubneyi* and of *F. hepatica* using larval forms have already been reported (Abrous *et al*., 1999b).

Experimental infections

Snail populations used for experimental infections originated from the commune of Saint Ours, department of Puy de Dôme (Massif Central, France), as the snail colonies in the department of Saône et Loire were too small. Snails were free from any natural trematode infections following regular sampling in May and June in this site, and from dissections of 50 adult snails on each date. Two hundred juvenile snails measuring 1 mm in height and 200 4 mm preadult snails for each freshwater species were collected in this commune at the end of June and were acclimatized to laboratory conditions for 48 h. Adult paramphistomes were collected in the rumen of cattle and placed in saline solution (NaCl, 0.9%; glucose, 0.45%) at 40°C for 4 h. Eggs of *F. hepatica* were collected from the gall bladders of heavily infected cattle. Both trematode eggs were washed in tap water and incubated for 20 days at 20°C in darkness.

Tables 3 and 4 give the number of experimental groups, the size of snails at exposure, and the type of infection (single or double) for *L. truncatula*, *L. ovata*, *L. palustris*, and *P. acuta*. Each experimental group consisted of 100 snails at miracidial exposure. Each snail was exposed to two miracidia of *P. daubneyi* for 4 h, or to one miracidium of *P. daubneyi* and then to one miracidium of *F. hepatica* 4 h later. Snails were subsequently kept for 30 days in open

Table 1. Pulmonate species found in six farms from the Saône et Loire department.

Farm n°	Geographic location	No. of snail habitats			
		L. truncatula	*L. ovata*	*L. palustris*	*Physa acuta*
1	Marigny	3	0	0	0
2	Le Breuil	2	0	0	2
3	La Guiche	2	1	0	0
4	Dompierre sous Sanvignes	2	1	0	0
5	Ciry	0	0	1	1
6	Châteauneuf	1	1	0	0

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Table 2. The trematode infection of *L. ovata*, *L. palustris*, *L. truncatula* and *Physa acuta* collected in July and September–October 1997 in six farms of the Saône et Loire department (eastern France).

Period of sampling	Snail species	No. of snails collected	No. of snails* with immature infection *Pd*	*Fh*	both	mature infection *Pd*	*Fh*	both	Prevalence of infection (%) *Pd*	*Fh*
July	*L. ovata*	47	3	4	0	0	0	0	6.4	8.5
	L. palustris	1	0	0	0	0	0	1	NC	NC
	L. truncatula	223	0	0	0	17	1	3	8.9	1.8
	P. acuta	59	3	0	0	0	0	0	5.1	0
September–October	*L. ovata*	61	2	0	0	0	0	0	3.3	0
	L. palustris	0	0	0	0	0	0	0	0	0
	L. truncatula	280	0	0	0	50	0	7	20.3	2.5
	P. acuta	171	4	0	0	0	0	0	2.3	0

Fh, *Fasciola hepatica*; *Pd*, *Paramphistomum daubneyi*; NC, not calculated.
*Other trematode species found in snails: *Notocotylus* sp. (in six *L. truncatula*) and unidentified species (in two *P. acuta*).

Table 3. Experimental infection of *L. truncatula, L. ovata*, *L. palustris*, and *Physa acuta,* in single infections with *Paramphistomum daubneyi*.

Snail species	Shell size at exposure (mm)	No. of surviving snails at day 35 p.e.	No. of snails with immature infection	mature infection	Prevalance of infection (%)
L. ovata	1	75	7	0	9.3
	4	84	0	0	0
L. palustris	1	77	9	0	11.6
	4	89	1	0	1.1
L. truncatula	1	62	6	48	87.0
	4	74	2	55	77.0
P. acuta	1	88	4	0	4.5
	4	99	1	0	1.0

boxes (1 m by 60 cm and 15 cm deep), with a density of 50 snails per box (Abrous *et al.*, 1999a). At day 35 post-exposure (p.e.), the surviving snails from each group were dissected to determine whether snails were infected, to determine the trematode species present, and to ascertain whether the most differentiated rediae of *P. daubneyi* and of *F. hepatica* were immature (with morulae and/or procercarial embryos) or contained procercariae and cercariae (mature infection).

Parameters studied

The prevalence of natural infection with *P. daubneyi* was calculated at each period of investigation (July, or September–October) using the ratio between the total number of singly and dually infected snails, and the total number of snails from each species collected in the six farms. A similar method was used to determine the prevalence of natural infections with *F. hepatica* in snails. In each experimental group, the respective prevalences were also calculated by the ratio between the total number of singly and dually infected snails, and that of surviving snails at day 35 p.e.

A comparison test of experimental frequencies (Stat-Itcf, 1988) was used to compare the prevalences of infection with *P. daubneyi* or with *F. hepatica*, and to establish levels of significance.

Results

Natural infections of snails

Table 2 gives, for each freshwater species, the number of snails harbouring *P. daubneyi* only, *F. hepatica* only, or both, and prevalences of infection. Paramphistome rediae and cercariae were found in *L. truncatula*, while *L. ovata* and *P. acuta* harboured only living sporocysts and immature rediae. Rediae and cercariae of *F. hepatica* were also noted in *L. truncatula*, while *L. ovata* contained only some living sporocysts and immature rediae. Lastly, the larval forms of both trematodes were found in a single *L. palustris* and in some *L. truncatula*. A significant difference ($P < 0.001$) between the prevalence of *P. daubneyi* infection noted in July and that found in September–October was noted only for *L. truncatula*. Another significant difference between the prevalences

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Table 4. Experimental infection of *Lymnaea truncatula, L. ovata*, *L. palustris*, and *Physa acuta,* in double infections with *Paramphistomum daubneyi* and *Fasciola hepatica*.

Snail species	Shell height at exposure (mm)	No. of surviving snails at day 35 p.e.	No. of snails with immature infection			No. of snails with mature infection			Prevalence of infection (%)	
			Pd	*Fh*	both	*Pd*	*Fh*	both	*Pd*	*Fh*
L. ovata	1	68	3	6	0	0	6	0	4.4	17.6
	4	75	0	1	0	0	0	0	0	1.3
L. palustris	1	81	7	5	1	1	4	0	11.1	12.3
	4	91	1	1	0	1	0	0	2.1	1.1
L. truncatula	1	71	4	7	1	22	31	7	47.8	64.7
	4	78	1	2	0	13	17	3	21.7	28.2
P. acuta	1	77	4	1	0	0	0	0	5.1	1.2
	4	94	0	0	0	0	0	0	0	0

Fh, *Fasciola hepatica*; *Pd*, *Paramphistomum daubneyi*.

of *P. daubneyi* infection was also noted: the infection rate recorded in September–October was higher ($P < 0.001$) in *L. truncatula* than in *L. ovata*.

Experimental infection of snails by P. daubneyi *only*

The number of infected snails and prevalence of infection are listed in table 3. The results differed in relation to the size of snails at exposure. In juvenile *L. truncatula*, the overall prevalence of *P. daubneyi* infection was 87% at day 35 p.e. (with 48 snails harbouring cercaria-containing rediae, and six snails with immature infection). Juvenile snails from the three other species harboured only some living sporocysts (4.5% of *P. acuta*), or some immature rediae (9.3% of *L. ovata*, and 11.6% of *L. palustris*). In preadult *L. truncatula*, the overall prevalence of infection was 77% (with 55 snails harbouring cercaria-containing rediae and two snails with immature rediae). Some living sporocysts were also noted in 1.1% of preadult *L. palustris* and in 1% of *P. acuta*. The prevalence of infection in *L. truncatula* was significantly higher ($P < 0.001$) than those recorded in the other three snail species. The other differences between these percentages were not significant, except in *L. palustris* for which prevalence recorded in 1 mm snails was greater ($P < 0.05$) than that found in 4 mm snails.

Experimental infection of snails by P. daubneyi *and* F. hepatica

In juvenile *L. truncatula* (table 4), the prevalence of *P. daubneyi* infection was 47.8% (26 snails with *P. daubneyi* only and eight snails with double infection), whereas that of *F. hepatica* infection was 64.7% (38 and eight snails, respectively). Most of these snails harboured cercaria-containing rediae (40.8% with *P. daubneyi*, 53.5% with *F. hepatica*). A similar finding was noted in juvenile *L. palustris*, however, the prevalences of infection (11.1% for *P. daubneyi*, and 12.3% for *F. hepatica*) as well as the percentage of snails harbouring cercaria-containing rediae (1.2% and 4.2% of snails, respectively) were lower. In juvenile *L. ovata*, mature and immature rediae of *F. hepatica* were detected in 17.6% of snails, while immature rediae of *P. daubneyi* were noted in 4.4% of snails. In juvenile *P. acuta*, only immature infections were detected (5.1% of snails with *P. daubneyi*, and 1.2% with *F. hepatica*).

In preadult *L. truncatula*, the global prevalence of *P. daubneyi* infection was 21.7% (14 snails with *P. daubneyi* only and three with double infection), whereas that of *F. hepatica* infection was 28.2% (19 and three snails, respectively). The results were more variable in the preadults of the other three species. In *L. palustris*, immature or mature rediae of *P. daubneyi* occurred in 2.1% of snails, whereas 1.1% of snails harboured only immature infections of *F. hepatica*. Immature rediae of *F. hepatica* were also detected in 1.3% of *L. ovata*. In *P. acuta*, all surviving snails were uninfected.

In *L. truncatula*, the prevalences of infection were significantly higher (*P. daubneyi*: $P < 0.001$; *F. hepatica*: $P < 0.001$) than those recorded in the other three snail species. A significant difference was also found between the prevalence of *P. daubneyi* infection and that of *F. hepatica* in 1 mm *L. truncatula* ($P < 0.05$), whereas no significant variation was noted in prevalences recorded in 4 mm snails. Most differences between the prevalences recorded in the other three snail species were not significant.

Discussion

The prevalence of *Paramphistomum daubneyi* (8.9% in July and 20.3% in September–October) in *L. truncatula* from the farms of the Saône et Loire department were similar to those found by Abrous *et al.* (1999b) in 11 highly infected farms of central France. However, they were clearly higher than the values recorded by Szmidt-Adjidé *et al.* (4.4% in 1994), or by Guichard (0.2% to 1.5% in 1997) in other sites of central France. This might arise from differences in local recruitment of *L. truncatula*, or to the absence of *P. daubneyi* infections in some regions of France. Conversely, the prevalences of *F. hepatica* infection in *L. truncatula* (1.8% in July and 2.5% in September–October) lay within the range reported by some workers in other sites of central France (Szmidt-Adjidé *et al.*, 1994: 7.3%; Rondelaud & Dreyfuss, 1997: 4.9–6.1%; Guichard, 1997: 2.2–5.3%).

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The ability of juvenile *L. ovata* and *L. palustris* to sustain the full larval development of *F. hepatica* is already known (Boray, 1978), whereas all experimental infections of *P. acuta* with this trematode were negative (Sampaio Xavier *et al.*, 1969; Barthe & Rondelaud, 1986). In contrast, our findings on natural or experimental infections of these snails by *P. daubneyi* warrant particular comments:

1. A single *L. palustris* dually infected by *P. daubneyi* and *F. hepatica* was found amongst snails collected in the field. Two snails harbouring *P. daubneyi* mature rediae were also noted in *L. palustris* dually exposed to both trematodes. This lymnaeid species must be regarded as a potential intermediate host in the life cycle of *P. daubneyi*, at least when this snail is co-infected with *F. hepatica*. This result agrees with those reported by Abrous *et al.* (1996) in *L. glabra* when snails were infected simultaneously by *P. daubneyi* and *F. hepatica*. This process would thus allow the larval development of *P. daubneyi* in a lymnaeid species and not necessarily as a single infection.

2. Sporocysts and immature rediae of *P. daubneyi* were found in *L. ovata* and *P. acuta*, in the field as well as in experimental infections. This finding contrasted with the results reported by Szmidt-Adjidé (1996) or by Abrous *et al.* (1998) in the same snail species of central France: according to these authors, no sporocysts and immature rediae were found in experimentally-infected juvenile and preadult snails. These differences might only be explained by variability in the susceptibility of *L. ovata* and *P. acuta* populations to *P. daubneyi* infection, as reported by Rondelaud (1993) in the *L. truncatula*–*F. hepatica* system: the prevalence of *F. hepatica* infection in snails increased with increasing frequency of encounter between snails and parasite in the field. The role of *L. ovata* and *P. acuta* as intermediate hosts in the life cycle of *P. daubneyi* is still uncertain and needs to be clarified by further studies on snails in farms known to have a history only of *P. daubneyi* infection in cattle.

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