A field study of natural infections in three freshwater snails with *Fasciola hepatica* and/or *Paramphistomum daubneyi* in central France

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Abstract

Natural infections of three freshwater snails with *Fasciola hepatica* and/or *Paramphistomum daubneyi* were studied during two periods in 1996 and 1997 (June–July and September–October) on 18 farms located in the departments of Vienne and Haute Vienne (central France), and known for low prevalences of *F. hepatica* infections in ruminants. A total of 1573 *Lymnaea glabra* and 1421 *L. truncatula* 6 mm high or more were collected in the meadows of 13 farms and dissected under laboratory conditions. Snails with single or concurrent infections of *F. hepatica* and/or *P. daubneyi* were found for each *Lymnaea* species. In *L. truncatula*, global prevalences of natural infections with *F. hepatica* (3.8% in June–July, and 3.6% in September–October) were significantly greater than those recorded for *P. daubneyi* (1.1% and 0.8%, respectively). In *L. glabra*, global prevalences of *F. hepatica* infections (0.4% in each investigation period) were significantly lower than those found in *L. truncatula*, whereas there were no significant differences between prevalences of *P. daubneyi* infections. A total of 2721 *Planorbis leucostoma* measuring at least 4 mm in diameter were collected in the meadows of the other five farms on which *L. truncatula* was absent. In these planorbids, global prevalences of natural infections with *F. hepatica* were 0.4% in each period of investigation. Contrary to fasciolosis, snail infections with *P. daubneyi* were not noted on all farms of the Vienne and Haute-Vienne departments. Natural single or concurrent infections with *F. hepatica* and/or *P. daubneyi* in *L. glabra* and a natural infection of *P. leucostoma* with *F. hepatica* were found in swampy meadows on acid soil.

Introduction

The snail *Lymnaea truncatula* is known to be the main intermediate host for the two trematodes: *Paramphistomum daubneyi* and *Fasciola hepatica*. As both parasites live in the same cattle hosts (Szmidt-Adjidé *et al.*, 2000), snails present in meadows grazed by these ruminants may be infected with one trematode, the other, or both. Concurrent infections of *L. truncatula* and *L. glabra* with these trematodes under laboratory conditions demonstrate that these snail species can simultaneously sustain full larval development of both trematodes and shedding of cercariae when the miracidial entry (one per trematode species) occurs in the same period of time (Abrous *et al.*, 1996; Augot *et al.*, 1996). In contrast, concurrent infections of other freshwater snails using the same protocol are negative, or allow only larval development of *F. hepatica* (*Planorbis leucostoma*: Abrous *et al.*, 1998) or of *P. daubneyi* (*Lymnaea fuscus* and *L. palustris*: Degueurce *et al.*, 1999; Abrous, 1999).

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These findings suggest that our previous knowledge needs to be questioned. It was necessary to confirm laboratory results with several studies on natural prevalences of infection with *F. hepatica* and *P. daubneyi* in freshwater snails. Previous investigations by Abrous *et al.* (1999) were carried out on *L. truncatula* and *L. glabra* inhabiting on 11 farms in central France, known for high prevalences of *F. hepatica* infection in ruminants. Snails infected with single or concurrent infections of *F. hepatica* and/or *P. daubneyi* were found in meadows inhabited by both *Lymnaea* species as well as in pastures colonized by each species only. When *L. glabra* was the only lymnaeid species in pastures, the prevalence of natural infections with *F. hepatica* or *P. daubneyi* ranged from 2.4% to 22.3%, but these values were clearly lower (less than 3%) when *L. glabra* were found with *L. truncatula* in the same meadows. The first aim of this paper was to verify if these low prevalences of natural infections recorded in *L. glabra* were also found in the pastures inhabited by both *Lymnaea* species on farms known for their low prevalences of fasciolosis in ruminants. The second aim was to determine whether natural infections of *P. leucostoma* with *F. hepatica* might exist on farms without *L. truncatula*. Investigations on 13 farms for *L. truncatula* and *L. glabra*, and on five farms for *P. leucostoma*, were carried out in 1996 or 1997 to accomplish these objectives.

Materials and methods

The first study was carried out on 13 farms located in the departments of Haute Vienne and Vienne (central France). Table 1 gives their principal characteristics. Sheep were reared on seven farms and cattle on the other six. These farms were known to have low prevalences of *F. hepatica* (less than 35% cattle or sheep infected). In contrast, paramphistomosis was only detected in ruminants from six farms (prevalence: $< 10\%$). On two farms, *L. truncatula* was the only species recovered. On the remaining 11 farms, *L. truncatula* and *L. glabra* were found in the same meadows. The 66 habitats of *L. truncatula* were confined to the peripheral extremities of open drainage furrows and ditches, whereas *L. glabra* lived in 25 isolated habitats in the lower levels of the same drainage networks. The second study (table 2) was carried out on five farms located in the same departments. The meadows were grazed by cattle (four herds) or sheep. The prevalence of fasciolosis ranged from 1.5 to 12.5%. Paramphistomosis was only found on two farms where

Table 1. Prevalence of infection with *Fasciola hepatica* and *Paramphistomum daubneyi* in cattle or sheep, and the species of *Lymnaea* found on 13 farms in central France in 1995 or 1997.

Farm no.	Farm or village name, and post code	Cattle or sheep	Prevalence (%) of infection (no. of ruminants examined)		No. of habitats with *Lymnaea*	
			F. hepatica (1995)	*P. daubneyi* (1997)[a]	*truncatula*	*glabra*
1	Les Fonts de Chaume, 86500	Sheep	5.3 (19)*	0 (35)*	5	2
2	L'Orgueil, Saint-Rémy, 86390	Sheep	11.3 (44)*	2.5 (79)*	2	2
3	Les Loges du Pont, 87300	Sheep	21.6 (37)*	0.8 (124)*	5	3
4	Le Bourg, 87300	Sheep	22.5 (31)*	0 (67)*	3	1
5	Pomereix, 87210	Cattle	10.0 (20)**	0 (21)*	11	5
6	La Poitevine, 87510	Cattle	33.3 (9)**	9.8 (41)*	3	2
7	Le Treuil, Landouge, 87100	Cattle	12.5 (16)*	3.3 (30)*	5	2
8	Las Goutarias, 87430	Cattle	6.0 (50)*	5.0 (50)*	2	3
9	La Garde, 86390	Sheep	6.2 (31)*	0 (77)*	7	0
10	Le Temple, 87400	Sheep	5.5 (36)*	0 (124)*	10	3
11	Sauviat-sur-Vige, 87400	Cattle	8.3 (12)*	0 (19)*	5	1
12	Les Souliers, 87500	Cattle	27.7 (18)*	7.5 (40)*	5	1
13	Poumier, 87500	Sheep	1.5 (64)*	0 (103)*	3	0

Methods used for parasite detection in ruminants: coproscopy* or serology**.
[a] Farm 6 (data from 1995).

Table 2. Prevalence of infection with *Fasciola hepatica* and *Paramphistomum daubneyi* in cattle or sheep, and the number of *Planorbis leucostoma* habitats found on five farms in central France in 1995, 1996, 1997 or 1998.

Farm no.	Farm or village name, and post code	Cattle or sheep	Prevalence (%) of infection (no. of ruminants examined and year of detection)		No. of planorbid habitats
			F. hepatica	*P. daubneyi*	
14	Chez le Mâcon, Saint-Rémy, 86390	Cattle	1.5 (66: 1996)*	1.5 (66: 1998)*	5
15	Maison-Celle, Saint-Rémy, 86390	Cattle	4.5 (44: 1995)*	0 (47: 1998)*	11
16	Saint-Pierre, 86290	Cattle	12.5 (24: 1997)**	1.5 (66: 1997)*	7
17	La Villatte, 87190	Cattle	5.0 (20: 1996)*	0 (33: 1998)*	4
18	Chez Vignan, 87330	Sheep	7.1 (42: 1997)*	0 (44: 1998)*	2

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L. truncatula was absent and *P. leucostoma* was the only freshwater snail. The habitats (two to eleven per farm) of *P. leucostoma* were located in the middle part and extremity of open drainage furrows.

Sampling of *L. truncatula* and *L. glabra* was carried out in 1996 on the first eight farms and in 1997 on farms 9 to 13. The planorbid snails were only collected in 1997. Each farm was only studied for one year, because the results found in snails led the breeders to request detection of trematode infections in their ruminants and also treatment. On these 18 farms snail sampling was performed in June–July and in September–October. The choice of these two periods was based on the fact that most cercarial sheddings from naturally-infected snails occurred during these months in central France and were thus risk periods for ruminants (Rondelaud & Dreyfuss, 1997). Snails collected were over 6 mm in height for *L. truncatula* and *L. glabra*, and over 4 mm in diameter for *P. leucostoma*. They were collected in the immersed zones of each area as well as in stagnant or shallow waters (no more than 5 cm in depth during the study period). The snail search in each habitat and subsequent collection were made by two persons, over 30 to 40 min.

Snails were dissected under the stereomicroscope to detect trematode larval forms and to classify infected snails into four groups: snails infected with *F. hepatica* only, *P. daubneyi* only, both, or snails infected by other trematode species. The most differentiated larval forms of *F. hepatica* and/or *P. daubneyi* found in most infected snails were cercaria-containing rediae and free cercariae; in the other snails, immature rediae were noted. Cercariae-containing rediae of *F. hepatica* possessed a well-developed pharynx (Augot *et al.*, 1998), collar rings and pairs of appendages in the third posterior part of their bodies (Thomas, 1883). In contrast, the rediae of *P. daubneyi* were shorter, with a small pharynx, and their bodies lacked a collar and appendages (Abrous *et al.*, 1997). Free-cercariae of *F. hepatica* were white-coloured and quick swimming, whereas those of *P. daubneyi* were dark brown and sluggish.

The respective prevalences of infections in each group of infected snails were calculated for each farm and study period. The global prevalences of natural infections with *F. hepatica* in each snail species were calculated by adding the number of snails harbouring only larval forms of *F. hepatica* and that of concurrently infected snails, and dividing it by the total number of snails examined. A similar method was used to determine global prevalences of natural infections with *P. daubneyi*. A comparison test of experimental frequencies (Stat-Itcf, 1988) was used to determine levels of significance.

Results

Infection of L. truncatula *with* F. hepatica *and* P. daubneyi

Snails harbouring *F. hepatica* only (table 3) were found on 12 of the 13 farms studied and prevalences ranged from 0 to 7.6% in June–July, and from 0 to 7.4% in September–October. Other *L. truncatula* harbouring *P. daubneyi* were only noted on six farms and prevalences ranged from 0 to 5.8% in June–July, and from 0 to 5.2% in September–October. Concurrently infected snails were scarce (two farms only) and the prevalence was less than 2.5%. No significant differences between prevalences recorded in the three groups of infected snails were found, whatever the mode of comparison.

Global prevalences of *F. hepatica* infections in *L. truncatula* were 3.8% (out of 1070 snails) in June–July and 3.6% (out of 354) in September–October. Those of *P. daubneyi* infection were lower: 1.1% in June–July and

Table 3. Prevalence of trematode infections in *Lymnaea truncatula* collected on 13 farms of the Haute Vienne and Vienne departments in 1996[a] or 1997[b].

Farm no.	June–July				September–October			
	No. of snails*	Prevalence (%) of snails infected with			No. of snails*	Prevalence (%) of snails infected with		
		F. hep.	*P. daub.*	both		*F. hep.*	*P. daub.*	both
1	93	3.2	0	0	27	3.7	0	0
2	37	2.7	2.7	0	11	0	0	0
3	76	6.5	1.3	1.3	19	5.2	5.2	0
4	49	2.0	0	0	27	7.4	0	0
5	201	3.9	0	0	54	7.4	0	0
6	51	3.9	5.8	0	9	0	0	0
7	77	2.5	1.2	0	16	0	0	0
8	136	2.9	2.2	0.7	42	4.7	2.3	2.3
9	91	7.6	0	0	47	2.1	0	0
10	74	4.0	0	0	23	0	0	0
11	115	1.7	0	0	61	1.6	0	0
12	43	2.3	2.3	0	11	0	0	0
13	27	0	0	0	7	0	0	0

[a] Farms 1–8.
[b] Farms 9–13.
*Other trematodes: *Haplometra cylindracea* (six snails), *Notocotylus* sp. (two snails), unidentified trematode with rediae (one snail).
F. hep., *Fasciola hepatica* only; *P. daub.*, *Paramphistomum daubneyi* only.

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Table 4. Prevalence of trematode infection in *Lymnaea glabra* collected on 11 farms of the Haute Vienne and Vienne departments in 1996[a] or 1997[b].

Farm no.	June–July				September–October			
	No. of snails*	Prevalence (%) of snails infected with			No. of snails*	Prevalence (%) of snails infected with		
		F. hep.	*P. daub.*	both		*F. hep.*	*P. daub.*	both
1	117	0	0	0	31	0	0	0
2	86	0	0	0	22	0	4.5	0
3	201	0.4	0.4	0	87	0	1.1	0
4	37	0	0	0	11	0	0	0
5	286	0	0	0	115	0	0	0
6	134	0	0	0	14	0	0	0
7	27	0	0	0	11	0	0	0
8	71	1.4	2.8	1.4	30	3.3	3.3	0
10	178	1.6	0	0	36	2.7	0	0
11	67	0	0	0	47	0	0	0
12	89	0	0	0	22	0	0	0

[a] Farms 1–8.
[b] Farms 9–13.
* Other trematodes: unidentified trematode with sporocysts (four snails), unidentified trematode with rediae (one snail).
F. hep., *Fasciola hepatica* only; *P. daub.*, *Paramphistomum daubneyi* only.

0.8% in September–October. When each trematode was considered separately, there was no significant difference between the prevalence recorded in June–July and that of September–October. In contrast, significant differences between the prevalence of *F. hepatica* infection and that of *P. daubneyi* were noted in June–July ($P < 0.05$) and September–October ($P < 0.01$).

Infection of L. glabra *with* F. hepatica *and* P. daubneyi

Snails harbouring *F. hepatica* only (table 4) were noted on three farms out of 11 in which specimens of *L. glabra* were examined and prevalences ranged from 0 to 1.6% in June–July, and from 0 to 3.3% in September–October. A similar finding was noted for snails infected with *P. daubneyi* but prevalences were slightly higher, i.e. 0–2.8% and 0–4.5%, respectively. A single snail harbouring both trematode larval forms was found in June–July (farm 8). As for *L. truncatula*, there were no significant differences between the prevalences recorded in the three groups of infected *L. glabra*.

Global prevalences of *F. hepatica* infections in *L. glabra* were 0.4% (out of 1293 snails) in June–July and 0.4% (out of 426) in September–October. Those of *P. daubneyi* infections were 0.3% and 0.7%, respectively. No significant differences between prevalences recorded in *L. glabra* were found.

Prevalences of *F. hepatica* infection were significantly higher in *L. truncatula* than in *L. glabra*, in June–July ($P < 0.01$) as well as in September–October ($P < 0.01$). In contrast, there were no significant differences between the prevalences of *P. daubneyi* infection recorded in the two *Lymnaea* species in either period of study.

Infection of P. leucostoma *with* F. hepatica

A total of 1841 snails in June–July and of 880 snails in September–October (table 5) were collected on the five

Table 5. Prevalence of trematode infection in *Planorbis leucostoma* collected on five farms of the Haute Vienne and Vienne departments in 1997.

Farm no.	June–July				September–October			
	No. of snails*	Prevalence (%) of snails infected with			No. of snails*	Prevalence (%) of snails infected with		
		F. hep.	*P. daub.*	both		*F. hep.*	*P. daub.*	both
14	331	0.9	0	0	116	0.8	0	0
15	244	0.8	0	0	87	0	0	0
16	527	0.7	0	0	244	0.8	0	0
17	412	0.4	0	0	317	0.3	0	0
18	327	0	0	0	116	0	0	0

* Unidentified trematode with rediae (one snail).
F. hep., *Fasciola hepatica* only; *P. daub.*, *Paramphistomum daubneyi* only.

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farms studied. Snails infected with *F. hepatica* only were found on four farms, and their prevalences ranged from 0 to 0.9% in June–July and from 0 to 0.8% in September–October (global prevalence of infection: 0.4% in each study period). No larval forms of *P. daubneyi* were detected in *P. leucostoma*.

Discussion

Global prevalences of *F. hepatica* infections in *L. truncatula* (2.5–3.8% according to the study period) were low when compared with values given by some authors in Spain (11.4%, Manga-Gonzalez *et al.*, 1991), in six departments of central France (15.5–17.2%, Abrous *et al.*, 1999), or in the Haute Vienne department (4.9–6.1%, Szmidt-Adjidé *et al.*, 1994, or 7.3%, Rondelaud & Dreyfuss, 1997). Comparison of the results reported in this paper with those given by Abrous *et al.* (1999) revealed a discrepancy between the prevalences of *F. hepatica* infection in *L. truncatula*. According to Abrous *et al.*, the prevalences of infection in the snail ranged from 4.6 to 33.0% when *L. truncatula* are collected on farms with 80–100% of cattle or sheep infected by *F. hepatica*. In view of this discrepancy, one may wonder whether the prevalence of *F. hepatica* infection in snails might not be related to that in the definitive host. This hypothesis could only be verified by the dissection of *L. truncatula* collected over some years on numerous farms from the same geographical area.

In contrast to *F. hepatica*, references on natural infections of *Lymnaea* snails with *P. daubneyi* were lower in number, as snail research began in 1992 only in central France. The paramphistome was only found on six farms in this work, and the prevalence of its infection in *L. truncatula* was low (0.5–11.1%) in comparison with the 4.4% on five other farms also situated in the Haute-Vienne department (Szmidt-Adjidé *et al.*, 1994). The most valid hypothesis to explain this difference would be to admit: (i) that this disease did not affect all bovine herds or sheep flocks in the Haute Vienne department; and (ii) that the intensity of *P. daubneyi* infection on each farm was different from that of fasciolosis. This last assumption is based mainly on the report by Szmidt-Adjidé *et al.* (2000) in the same department as above, where the mean prevalence of *P. daubneyi* infection in slaughtered cattle was higher than that of *F. hepatica* infection (24.1% out of 1434 instead of 7.8% for fasciolosis).

Low prevalences of natural infections recorded in *L. glabra* (0.4% for *F. hepatica*, 0.3–0.7% for *P. daubneyi*) could readily be explained by the results obtained by Abrous *et al.* (1999) when *L. truncatula* and *L. glabra* lived in the same swampy meadows on acid soil: when *L. truncatula* was present, prevalences of infections by either of the trematodes in *L. glabra* were very low (or nil), while they reached 8–10% when *L. glabra* was the single *Lymnaea* species in meadows.

The prevalence of *F. hepatica* infection was also low in *P. leucostoma* (0.4%) but this value was sufficient to explain the presence of fasciolosis on farms where *L. truncatula* was never found despite numerous investigations in these meadows over a 20-year period (Abrous *et al.*, 1998). The presence of paramphistomosis in two farms and the absence of *P. daubneyi* larvae in the planorbid snails were still open to question. To resolve this problem, further studies are necessary to understand how *P. leucostoma* might sustain the local larval development of this trematode.

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