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Short note

Infection of *Lymnaea truncatula* and *Lymnaea glabra* by *Fasciola hepatica* and *Paramphistomum daubneyi* in farms of central France

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Abstract – Natural infection of *Lymnaea glabra* and *Lymnaea truncatula* by *Fasciola hepatica* and/or *Paramphistomum daubneyi* was studied at two periods of risk (June–July and September–October) in 11 French farms known for their high prevalences of *F. hepatica* infection in ruminants. A total of 1 778 *L. truncatula* and 2 396 *L. glabra* measuring 6 mm or more in height were collected to determine the prevalence of natural infection with *F. hepatica* and *P. daubneyi*. The role of four factors, i.e. season, snail co-infection (*F. hepatica–P. daubneyi*), ruminant host and the existence of single or mixed intermediate host communities, was investigated. There were no differences in prevalences between the two risk periods. Co-infections in *L. glabra* were more frequent than expected. The prevalences of infection with *F. hepatica* or *P. daubneyi* in *L. glabra* were higher in farms rearing sheep than in farms rearing cattle. A similar finding was also noted for *L. truncatula* infected with *P. daubneyi* only. *L. glabra* was a much better intermediate host for *F. hepatica* and *P. daubneyi* when it was the only available snail, possibly indicating an adaptation of parasites to their less usual host in local conditions. The two trematodes preferably developed in *L. truncatula* rather than in *L. glabra* when both host species lived in the same places. © Inra/Elsevier, Paris.

***Fasciola hepatica* / *Lymnaea glabra* / *Lymnaea truncatula* / *Paramphistomum daubneyi* / natural infection**

Résumé – L'infestation de *Lymnaea truncatula* et de *Lymnaea glabra* par *Fasciola hepatica* et *Paramphistomum daubneyi* dans des fermes du centre de la France. L'infestation naturelle de *Lymnaea glabra* et de *Lymnaea truncatula* par *Fasciola hepatica* et/ou *Paramphistomum daubneyi* a été

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étudiée lors de deux périodes à risque (juin–juillet et septembre–octobre) dans 11 fermes françaises ayant des prévalences élevées de fasciolose chez les ruminants. Des limnées mesurant 6 mm de hauteur et plus (1778 *L. truncatula*, 2396 *L. glabra*) y ont été récoltées afin de déterminer la prévalence de leur infestation naturelle. Les résultats ont été analysés en tenant compte de quatre facteurs, à savoir la saison, la co-infestation des mollusques par *F. hepatica* et *P. daubneyi*, l'espèce du ruminant et le type de peuplement pour les limnées (populations isolées ou colonies mixtes des deux espèces). Il n'y avait pas de différence entre les prévalences notées pour les deux périodes à risque. Les co-infestations étaient plus fréquentes que prévues chez *L. glabra*. Les prévalences notées chez *L. glabra* étaient plus élevées dans les prairies pâturées par les moutons que dans les pacages à bovins, quelle que soit l'espèce du trématode. Le même fait a été noté également chez *L. truncatula* infestée par *P. daubneyi*. *L. glabra* était un meilleur hôte intermédiaire pour *F. hepatica* et *P. daubneyi* lorsqu'il était le seul mollusque disponible, ce qui indique la possibilité d'une adaptation des deux trématodes à cet hôte inhabituel dans des conditions locales. *Fasciola hepatica* et *P. daubneyi* se sont développés préférentiellement chez *L. truncatula* plutôt que chez *L. glabra* lorsque les deux hôtes vivaient dans les mêmes pâtures. © Inra/Elsevier, Paris.

***Fasciola hepatica* / *Lymnaea glabra* / *Lymnaea truncatula* / *Paramphistomum daubneyi* / infestation naturelle**

1. INTRODUCTION

In the centre of France, cattle are frequently infected by two trematodes: *Fasciola hepatica* and *Paramphistomum daubneyi*. The periods of high risk are June–July and September–October, when the majority of cercarial shedding by the snail intermediate host occurs. The two trematodes use *Lymnaea truncatula* as an intermediate host (*F. hepatica* [9], *P. daubneyi* [3, 6]). *Lymnaea glabra* is also frequently found in the pastures. It may act as an intermediate host for both trematodes in experimental conditions [1] and is infected with *F. hepatica* under natural conditions when *L. truncatula* is not found [2], possibly owing to a local adaptation of the parasite. Its susceptibility could possibly be increased when dually infected with *F. hepatica* and *P. daubneyi* as shown in experimental infections [1]. We have, in natural conditions, compared the infection of *L. truncatula* and *L. glabra* in farms where *F. hepatica* and *P. daubneyi* are available at two periods of high risk (interaction between parasites). Farms were chosen because of the presence of isolated populations or mixed communities of *L. truncatula* and *L. glabra* (interaction between hosts upon parasite infection).

2. MATERIALS AND METHODS

The 11 farms were located in seven different French departments and were grazed by cattle (six farms) or sheep (five farms). *Table I* gives their principal characteristics. These farms were known to have high prevalences of *F. hepatica* (> 70 % of infected cattle or sheep) and low to moderate prevalences of *P. daubneyi*. On four farms (14 habitats), *L. glabra* was the only species recovered. On another four farms (31 habitats), *L. truncatula* was found alone. Both *Lymnaea* species were observed on the last three farms (11 habitats with *L. truncatula* and four with *L. glabra*).

Snail sampling was performed in the different habitats located in the 11 farms in June–July and in September–October 1996. 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 thus constituted risk periods for ruminants. As the larval forms of *F. hepatica* are more frequent in adults than in juvenile *L. truncatula* [8], only snails measuring 6 mm or more in height were searched visually and collected at each investigation period. They were dissected under a stereomicroscope to detect trematode larval forms and to classify infected snails into four groups: snails infected with *F. hepatica* only, *P. daubneyi* only, or both, and snails infected by other trematode species. Cercaria-containing rediae of *F. hepatica* possessed well-developed pharynxes, collar rings and pairs of appendages

Table I. *Lymnaea* species found in 11 farms from the centre of France, with records of infection with *Fasciola hepatica* and *Paramphistomum daubneyi* in sheep and cattle.

Farm no.	Geographic location (department)	Sheep or cattle	Number of *Lymnaea* habitats		Prevalence[a] of ruminant infection (%)	
			L. truncatula	*L. glabra*	*F. hepatica*	*P. daubneyi*
1	Boisbuchet, Lessac (Charente)	cattle	11	0	84.5	6.4[b]
2	La Valette, St-Victurnien (Haute-Vienne)	sheep	8	0	72.8	16.0
3	Chézeau-Chrétien, Migné (Indre)	cattle	5	0	96.4	8.5
4	Le Mas del Sol, Rueyres (Lot)	sheep	7	0	70.5	75.0
5	Le Bourg, Sainte-Féréole (Corrèze)	sheep	0	4	82.7	24.5
6	Les Ferrières, Seilhac (Corrèze)	sheep	0	2	83.5	43.3
7	Champsiaux, La Meyze (Haute-Vienne)	cattle	0	5	84.5	2.7
8	Le Cluzeau, Rancon (Haute-Vienne)	cattle	0	3	77.2	11.5
9	La Ponterie, Objat (Corrèze)	cattle	5	1	92.5	18.5[b]
10	La Ferrière-en-Parthenay (Deux-Sèvres)	cattle	2	1	100	86.3
11	La Vergne, Mézières-sur-Issoire (Haute-Vienne)	sheep	4	2	85.5	17.7

[a] These records were obtained by the examination of faecal samples (*F. hepatica*, *P. daubneyi*), and by the livers refused for fasciolosis originating from these farms in 1996.
[b] These prevalences were determined in 1997.

in the third posterior parts of their bodies [9]. In contrast, the rediae of *P. daubneyi* were shorter, with small pharynxes, and their bodies had no collar or appendages [6]. Free cercariae of *F. hepatica* were white-coloured, quick swimming organisms [9]. The bodies of *P. daubneyi* cercariae were dark brown and their swimming was sluggish [6].

The respective prevalences of natural infection in each group of infected snails were calculated in each farm and at each investigation period. A three-way variance analysis (ANOVA) and the Kruskall-Wallis test were used to compare the prevalences of infection with *F. hepatica* only, *P. daubneyi* only, or both, and to establish the levels of significance.

3. RESULTS

Table II gives the prevalences of natural infection in each farm when *L. truncatula* and/or *L. glabra* harboured *F. hepatica* only, *P. daubneyi* only, or both trematodes. When the pastures were colonized by *L. truncatula* only, the prevalence of infection ranged from 4.6 to 33.0 % for *F. hepatica*, from 0 to 20.6 % for *P. daubneyi*, and from 0 to 5.9 % for both trematodes. When the pastures were inhabited by *L. glabra* only, the range of prevalences was 4.2–14.5 %, 2.4–23.3 % and 1.2–9.3 %, respectively. When both *Lymnaea* species lived on the same pastures,

Table II. The trematode infection of *Lymnaea truncatula* and *L. glabra* collected in June–July and September–October 1996 in 11 farms of the centre of France.

Habitats	Farm	June–July				September–October			
with	no.	Number of snails collected	Prevalence (%) of snails infected with			Number of snails collected	Prevalence (%) of snails infected by		
			Fh	*Pd*	both		*Fh*	*Pd*	both
L.	1	78	21.7	6.4	1.2	33	33.0	10.0	0
truncatula	2	62	14.5	11.2	3.2	17	11.7	11.7	5.8
only[a]	3	347	4.6	2.5	0	87	13.7	0	0
	4	587	14.8	20.6	5.9	142	12.6	17.6	2.8
L.	5	343	7.8	3.2	2.6	78	11.5	14.1	1.2
glabra	6	289	4.8	2.4	9.3	103	14.5	23.3	1.9
only[b]	7	257	4.2	6.2	1.9	86	6.9	8.1	2.3
	8	156	4.4	7.0	1.9	58	6.8	8.6	1.7
L.	9	67	10.4	7.4	2.9	17	17.6	11.7	0
truncatula[a]	10	178	10.1	15.1	6.1	45	15.5	11.1	4.4
and	11	91	15.3	7.6	6.5	27	11.1	11.1	14.8
L.	9	131	0	0	0	54	0	0	0
glabra	10	455	2.4	0.2	1.7	187	1.6	2.6	0
	11	141	0	0	0	58	0	0	0

[a] Other trematode species found in *L. truncatula*: *Haplometra cylindracea* (farm no. 4: two snails in June–July and one snail in September–October; farm no. 11: one snail in June–July). *Notocotylus* sp. (farm no. 10: three snails in June–July and one snail in September–October).

[b] Other trematode species found in *L. glabra*: one unidentified species, having rediae and echinostome-type cercariae (farm no. 5: 11 snails in June–July and one snail in September–October).

Abbreviations: *Fh* (with *Fasciola hepatica* only). *Pd* (with *Paramphistomum daubneyi* only).

the prevalences of *L. truncatula* did not significantly differ from those found in the habitats colonized by *L. truncatula* only, whereas the prevalences found in *L. glabra* were lower than 3 %. Other trematode species were also noted in eight *L. truncatula* (*Haplometra cylindracea*: from 0 to 0.7 %; *Notocotylus* sp.: from 0 to 1.6 %) and in 12 *L. glabra* (an unidentified species: from 0 to 3.4 %).

The actual prevalence of infection in dually infected snails (*table II*) was compared to the prevalence that might be expected using the following formula: [(prevalence of *F. hepatica* infection only + prevalence of dual infection) × (prevalence of *P. daubneyi* infection only + prevalence of dual infection)]. Co-infections were more frequent than expected only in *L. glabra* ($P = 0.01$). The prevalences were also compared to determine the eventual influence of the following three factors: the investigation period (June–July, or September–October), the type of ruminants in the farms (cattle or sheep), and the type of community for *Lymnaea* species (isolated populations or mixed species). The prevalences for both trematodes did not differ significantly when the investigation period was considered. The prevalences of infection in *L. glabra* collected in farms rearing sheep were higher than the corresponding percentages obtained in farms rearing cattle. This variation was significant using ANOVA in *F. hepatica*-infected *L. glabra* ($F = 5.75$; $P = 0.04$) and in *P. daubneyi*-infected *L. glabra* ($F = 8.8$; $P = 0.01$). Higher prevalence of *P. daubneyi* infection (Kruskall-Wallis test: $P = 0.008$; ANOVA: $F = 8.7$; $P = 0.01$) was also noted in *L. truncatula* when collected in pastures grazed by sheep. Lastly, *L. glabra* was more often infected by both parasites (*F. hepatica*: $P = 0.002$; *P. daubneyi*: $P = 0.002$) when it was the only available snail in the pastures.

4. DISCUSSION

Works by Roberts [4], Rondelaud [5] and Smith [7] demonstrated that in western Europe, cercariae of *F. hepatica* were shed by spring-born *L. truncatula* in June or July, and in September–October after habitat aestivation. The snails collected during these two periods belonged to the same generation and had similar prevalences of infection, for *F. hepatica* as well as for *P. daubneyi*.

The prevalence of natural infection with *F. hepatica* or *P. daubneyi* in *L. glabra* was low when this snail species lived with *L. truncatula* in the same pastures. However, it was clearly higher when *L. glabra* was the only lymnaeid species in the habitat. This agrees with the results reported by Bouix-Busson and Rondelaud [2]. They stated that the prevalence of *F. hepatica* infection was 2 % in juvenile *L. glabra* when this species was associated with *L. truncatula*, whereas it was 11 % when *L. glabra* was the only lymnaeid species in the pastures. This could be the result of a local adaptation between host and parasite: when only the less susceptible *L. glabra* is available, the population of *F. hepatica* must adapt to this host or disappear.

Our results also demonstrated that prevalences of *P. daubneyi* infection in *L. glabra* and *L. truncatula* were higher when these snails lived in pastures grazed by sheep. One can only speculate that there is a higher possible infection of *P. daubneyi* in sheep which might result in subsequent higher snail infection. Only comparative experimental infections in sheep and cattle could give support or infirm this speculation.

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