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# UNUSUAL SNAIL SPECIES INVOLVED IN THE TRANSMISSION OF *FASCIOLA HEPATICA* IN WATERCRESS BEDS IN CENTRAL FRANCE

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**Summary:**

Four freshwater pulmonate species (*Lymnaea ovata, L. stagnalis, Physa acuta, Planorbis leucostoma*) were living in several watercress beds known for their relationships with human cases of fasciolosis, whereas *L. truncatula* was never found. The aims of these studies were to determine the prevalence of natural infections with *Fasciola hepatica* in snails and to verify if these species might ensure the full larval development of this trematode (with cercarial shedding) when they were experimentally subjected to *F. hepatica* only, or to co-infections with an other trematode species. Investigations were so carried out in six snail populations living in watercress beds (including three for *P. acuta*) and in four others originating from three brooks or a pond (as controls). Snails naturally infected with *F. hepatica* were found in two watercress beds inhabited by *L. ovata* (prevalence of infection: 1.4 %) and *P. leucostoma* (0.1 %), respectively. The *L. ovata* from the watercress bed could be infected at a higher size than those from the control population and the prevalence of this infection was greater in the bed population. Similar findings were noted for *L. stagnalis*. Despite single or dual infections, the results obtained with the four populations of *P. acuta* were unsuccessful. In contrast, the co-infections of young *P. leucostoma* with *Paramphistomum daubneyi* and *F. hepatica* resulted in the shedding of some *F. hepatica* cercariae. According to the authors, the occurrence of fasciolosis in these watercress beds would be the consequence of frequent natural encounters between parasite and snails (*L. ovata, L. stagnalis*), or of co-infections with *P. daubneyi* and *F. hepatica* (*P. leucostoma*). In watercress beds only colonized by *P. acuta*, a lymnaeid species would have ensured the larval development of *F. hepatica* but it would have been eliminated by *P. acuta*, as this last species was known to be invasive and could colonize open drainage ditches on siliceous soil.

**KEY WORDS :** *Fasciola hepatica, Lymnaea ovata, L. stagnalis, Paramphistomum daubneyi, Physa acuta, Planorbis leucostoma*, France, parasitic infections, watercress bed.

***Résumé :*** MOLLUSQUES INHABITUELS IMPLIQUÉS DANS LA TRANSMISSION DE *FASCIOLA HEPATICA* DANS DES CRESSONNIÈRES DU CENTRE DE LA FRANCE

*Quatre espèces de mollusques aquatiques* (Lymnaea ovata, L. stagnalis, Physa acuta, Planorbis leucostoma) *se rencontrent dans plusieurs cressonnières à l'origine de cas humains de distomatose alors que* Lymnaea truncatula *n'y vit pas. Le but de ces études est de déterminer la prévalence de leur infestation naturelle avec* Fasciola hepatica *et de vérifier si ces espèces peuvent assurer le développement larvaire complet (avec émission de cercaires) de ce Digène lorsqu'elles sont soumises à* F. hepatica, *ou à des co-infestations avec un autre Digène. Les investigations ont donc été réalisées sur six populations de cressonnières (dont trois pour* P. acuta) *et sur quatre autres colonies (comme témoins) vivant dans des ruisseaux ou un étang. Des mollusques infestés naturellement par* F. hepatica *ont été trouvés dans deux cressonnières colonisées par* L. ovata *(prévalence de l'infestation: 1,4 %) et* P. leucostoma *(0,1 %). Si l'on soumet* L. ovata *à des expositions simples ou répétitives avec* F. hepatica, *on constate que la gamme des hauteurs de coquilles pour lesquelles l'espèce est capable de s'infester est plus étendue et que la prévalence de l'infestation est plus élevée pour la population de la cressonnière. Des résultats allant dans le même sens ont été notés chez* L. stagnalis. *Malgré des infestations simples ou doubles, les résultats obtenus dans les quatre populations de* P. acuta *se sont révélés négatifs. A l'inverse, la co-infestation de jeunes* P. leucostoma *avec* Paramphistomum daubneyi *et* F. hepatica *a permis l'émission de quelques cercaires de* F. hepatica. *D'après les auteurs, la survenue de la fasciolose dans ces cressonnières dépendrait de rencontres fréquentes entre le mollusque et* F. hepatica (L. ovata, L. stagnalis) *ou de co-infestations avec* P. daubneyi (P. leucostoma). *Dans les cressonnières peuplées seulement par* P. acuta, *le développement larvaire de* F. hepatica *aurait été assuré par une limnée mais celle-ci aurait été éliminée par* P. acuta *car cette espèce est connue pour être invasive et est capable de remonter les rigoles de drainage superficiel sur sol siliceux.*

***MOTS CLÉS :*** Fasciola hepatica, Lymnaea ovata, L. stagnalis, Paramphistomum daubneyi, Physa acuta, Planorbis leucostoma, *cressonnière, France, infestation parasitaire.*

## INTRODUCTION

Numerous wild or home-grown watercress beds, located in the region of Limousin (central France), are the cause of human cases of fasciolosis. Several snail species can be found in these watering places: 84.7 % of them are colonized by *Lymnaea truncatula*, 6.4 % by *L. glabra*, 1.7 % by another species of *Lymnaea*, and 7.2% by *L. truncatula* and an other lymnaeid species (Rondelaud *et al.*, 2000). If *L. truncatula* is the main intermediate host of *Fasciola hepatica* in western Europe (Mas Coma *et al.*, 1999), the finding of *Fasciola* larval forms in naturally-infected *L. glabra* demonstrates the role of this species as a natural snail host of this trematode in the region of Limousin (Rondelaud & Dreyfuss, 1998).

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Among the 239 watercress beds recorded in central France (Rondelaud *et al.*, 2000; Rondelaud, unpublished data), six places only were inhabited by a single freshwater snail species: *Lymnaea ovata* (one site), *L. stagnalis* (one place), *Physa acuta* (three sites), or *Planorbis leucostoma* (one place). The two former species are already known for their ability to ensure the full larval development of *F. hepatica* when the experimental infection was performed using very young snails (Boray, 1978, for example). In contrast, no successful infections were obtained with *P. acuta* or *P. leucostoma* when exposed to the miracidia of this trematode. As the six watercress beds were the source of seven cases of human fasciolosis (Table I), the three following questions had arisen: might these four snail species develop a natural infection with *F. hepatica* when *L. truncatula* was not present ? Was this ability of these four species to develop an infection with *F. hepatica* limited to snail populations living in watercress beds, or did it concern all populations of these species? Were these unusual species susceptible in relation to *F. hepatica* infection, an other trematode infection, and origin or age of snail ? To answer these questions, field investigations in the six watercress beds and experimental infections of snails with *F. hepatica* and/or an other trematode species were performed. The results were compared with those we have obtained for four other populations of snails (one population per species), as they were not known for their role in the transmission of human or animal fasciolosis, and were so considered as controls in this study.

## MATERIALS AND METHODS

Ten populations of freshwater pulmonate snails were used. Six of them were living in watering places whose watercress had been eaten by several persons (Table I) before the development of fasciolosis symptoms. These six watercress beds had been identified by these persons during an epidemiological survey performed in the year or the two years which had followed the detection of their parasitosis (Rondelaud, 1980; Rondelaud *et al.*, 2001). Table I indicates the principal characteristics of these sites. Water was permanently running in these six sites throughout the year. In spite of repeated investigations twice a year from 1978 to 2000, *L. truncatula* was never found in these beds, and the community of snails (*L. ovata*, *L. stagnalis*, *P. acuta*, or *P. leucostoma*) living in each place had not changed in its composition between the year of the epidemiological survey (see Table I) and the past 22 years. Low prevalences of *F. hepatica* infections were recorded in the six cattle herds, which grazed in the nearby of these beds. Three populations of uninfected snails were used as controls. They originated from brooks in the department of Haute-Vienne and were living in the communes of Limoges (*L. ovata*, *P. acuta*) and Tersannes (*P. leucostoma*), while the last population (*L. stagnalis*) inhabited a small pond, in the commune of Thenay, department of Indre.

Samples of snails measuring 3 mm or more in height were collected from each watercress bed in October-November 1999, March 2000, and in June-July 2000.

| Location of the bed: commune (and locality) | Description (and size of the bed in m²) | Snails species* (and number of snails on March 1999) | Potential contamination by mammalians infected with *Fasciola hepatica*† | Number of human cases (and year) |
|---|---|---|---|---|
| Beaune-les-Mines (Le Bourg) | Spring in a meadow (2.5) | *Lymnaea ovata* (104) | Cattle and lagomorphs | 1 (1968) |
| Arnac-la-Poste (Les Tribelles) | Overflowing ditch near a pond (4.7) | *L. stagnalis* (33) | Cattle | 2 (1977) |
| Magnac-Bourg (Le Monceau) | Spring in a ditch along the road D 82 (6.3) | *Physa acuta* (84) | Cattle | 1 (1981) |
| Saint-Sornin-Leulac (La Zaphix) | Pool with spring in a meadow (5.2) | *Physa acuta* (33) | Cattle | 1 (1988) |
| Thiat (Bachellerie) | Spring in a meadow (7.2) | *Physa acuta* (67) | Cattle and lagomorphs | 1 (1969) |
| Saint-Léger-Magnazeix (Les Sechères) | Spring in a meadow (4.6) | *Planorbis leucostoma* (185) | Cattle | 1 (1974) |

* *Lymnaea truncatula* was never found in these six stations.

† Infections rates of cattle with *F. hepatica* (1997 or 1998): Arnac-la-Poste (33.3 %), Beaune-les-Mines (8.7 %), Magnac-Bourg (15.0 %), Saint-Léger-Magnazeix (24.5 %), Saint-Sornin-Leulac (7.7 %), Thiat (22.3 %). The prevalences of *F. hepatica* infections in lagomorphs were not determined.

Table I. – Geographic location and main characteristics of the six wild watercress beds studied in the department of Haute-Vienne (central France).

Snails were transported to the laboratory and were dissected under a stereomicroscope to find any trematode larval forms. Naturally-infected snails were counted in each sample, taking into account trematode species (*F. hepatica*, *Haplometra cylindracea*, *Paramphistomum daubneyi*, or an other helminth). The larval forms of the first three helminth species were particularly searched, as *F. hepatica*, *H. cylindracea*, and *P. daubneyi* were the most frequent Digenea found in snails from the watercress beds of central France (Rondelaud *et al.*, 2001).

Other samples of 15-20 adult snails each were also collected from each population in October 1999 and were maintained in aquaria (at 20° C) under laboratory conditions until they had laid eggs. The progeny of these snails measuring 1, 2, or 4 mm in height were subsequently used for miracidial exposure. Eggs of *F. hepatica* were collected from the gall bladders of heavily infected cattle. To obtain eggs of *P. daubneyi*, adult worms were collected from the paunch of the same ruminants and placed in a saline solution (NaCl, 0.9 %; glucose, 0.45 %) at 40° C for four hours. The choice of local and infected cattle to obtain the eggs of both aforementioned trematodes was determined in relation to the presence of cattle around the six watercress beds studied. The eggs of *Haplometra cylindracea* were released by naturally-infected frogs (*Rana esculenta*, or *R. temporaria*). The three types of eggs were washed several times with spring water and incubated in complete darkness at 20° C for 20 days.

Four experiments were performed using a total of 64 groups (100 snails per group). The species and populations of snails used for each experiment, the number and size of groups, and the details of miracidial exposure are given in Table II. The choice of a 24-hour (repeated infections with *F. hepatica*) or a 4-hour (bispecific infections with another trematode, then with *F. hepatica*) interval between two successive miracidial exposures was based on the results that Augot (1998), Augot *et al.* (1996) had reported with similar infections of *L. truncatula*. After the last exposure, the snails from the 64 groups were subsequently reared for 30 days in closed-circuit aquaria, with five snails per litre of water (Hourdin *et al.*, 1993). Aquaria were maintained under constant conditions at 20° C, with a diurnal photophase of 3,000-4,000 lux light intensity. At day 30 post-exposure (p.e.), the surviving snails from the 64 groups were individually isolated in 35-mm diameter petri dishes, with 2-3 mL of spring water and a piece of lettuce. The dishes were maintained under the same conditions as the aquaria. Every day, the metacercariae were counted and removed from the dishes, and the water changed. This surveillance was performed until snail death. Routine postmortem dissection of cadavers was thus performed to identify trematode species and recognize the most differentiated larval stage.

The prevalence of natural infection in snails after their collection from the field was calculated using the ratio between the number of snails harbouring larval forms of a trematode species and that of snails collected in a watercress bed. Three other parameters were studied in experimental infections: the survival rate at day 30 p.e., the prevalence of experimental

| Snails species and population | Type of infection (and trematodes) | Number of groups per population* | Details of miracidial exposure |
|---|---|---|---|
| The 10 populations | Single (*F. hepatica*) | 3 groups:<br>1-mm high snails<br>2-mm high snails<br>4-mm high snails | 2 miracidia per snail (1 exposure) |
| *Physa acuta* and *Planorbis leucostoma* (6 populations) | Repeated (*F. hepatica*) | 3 groups:†<br>2 exposures<br>3 exposures<br>4 exposures | 2 miracidia per snail and per exposure. A 24-hour interval separated 2 successive exposures |
| *P. acuta* and *P. leucostoma* (4 populations from watercress beds) | Single (another trematode species) | 2 groups:†<br>*Haplometra cylindracea* only (1 exposure)<br>*Paramphistomum daubneyi* only (1 exposure) | 2 miracidia per snail (1 exposure) |
| | Double (another trematode + *F. hepatica*) | 2 groups:†<br>*H. cylindracea* + *F. hepatica* (2 successive exposures)<br>*P. daubneyi* + *F. hepatica* (2 successive exposures) | 2 miracidia per snail and per exposure. A 4-hour interval separated 2 successive exposures |

* 100 snails per group at miracidial exposure.
† Experiences performed using 1-mm high snails.

Table II. – Characteristics of the 64 groups of snails experimentally infected with *Fasciola hepatica* and/or an other trematode species.

| Geographic location | Species | Number of snails collected* (and number of snails harbouring larval forms of *Fasciola hepatica*) | | | Prevalence (%) of natural infection with *F. hepatica* |
|---|---|---|---|---|---|
| | | October-November | March | May-june | |
| Beaune-les-Mines | *Lymnaea ovata* | 104 (3) | 79 (2) | 244 (1) | 1.4 |
| Arnac-la-Poste | *L. stagnalis* | 45 (0) | 31 (0) | 122 (0) | 0 |
| Magnac-Bourg | *Physa acuta* | 98 (2†) | 56 (0) | 147 (1†) | 0.6 |
| Saint-Sornin-Leulac | | 78 (1†) | 34 (1†) | 97 (0) | 0.9 |
| Thiat | | 27 (0) | 44 (0) | 67 (0) | 0 |
| Saint-Léger-Magnazeix | *Planorbis leucostoma* | 123 (0) | 123 (0) | 211 (1) | 0.1 |

* The snails collected from the sites were 3 mm and more in height.
† Live (4 snails) or degenerated (1 snail) sporocysts of *F. hepatica*.

Table III. – Numerical distribution of naturally-infected snails with *Fasciola hepatica* in six watercress beds of the Limousin region between October 1999 and June 2000.

| Snail species and population | Size (mm) of snails at miracidial exposure | Number of snails | | | Number of cercariae per snail (means ± S.D.) |
|---|---|---|---|---|---|
| | | At day 30 p.e. | With larvae (and prevalence of infection in %) | With cercarial shedding | |
| *Lymnaea ovata*, | 1 | 57 | 27 (47.3) | 24 | 15.3 ± 11.2 |
| Beaune-les-Mines, | 2 | 64 | 16 (25.0) | 12 | 27.4 ± 18.7 |
| watercress bed | 4 | 78 | 11 (14.1) | 8 | 42.5 ± 21.3 |
| *L. ovata*, | 1 | 39 | 9 (23;0) | 4 | 10.3 ± 11.7 |
| Limoges, | 2 | 35 | 0 | 0 | 0 |
| control | 4 | 52 | 0 | 0 | 0 |
| *L. stagnalis*, | 1 | 37 | 14 (37.8) | 5 | 16.6 ± 9.8 |
| Arnac-la-Poste, | 2 | 46 | 2 (4.3) | 1 | 24 |
| watercress bed | 4 | 55 | 0 | 0 | 0 |
| *L. stagnalis*, | 1 | 31 | 5 (16.1) | 0 | 0 |
| Thenay, | 2 | 49 | 0 | 0 | 0 |
| control | 4 | 46 | 0 | 0 | 0 |
| *Physa acuta*, | 1 | 58 | 3 (5.1)* | 0 | 0 |
| Magnac-Bourg, | 2 | 68 | 2 (2.9)* | 0 | 0 |
| watercress bed | 4 | 91 | 0 | 0 | 0 |
| *P. acuta*, | 1 | 62 | 5 (8.0)* | 0 | 0 |
| Saint-Sornin-Leulac, | 2 | 72 | 1 (1.3)* | 0 | 0 |
| watercress bed | 4 | 88 | 0 | 0 | 0 |
| *P. acuta*, | 1 | 64 | 1 (1.5)† | 0 | 0 |
| Thiat, | 2 | 67 | 0 | 0 | 0 |
| watercress bed | 4 | 85 | 0 | 0 | 0 |
| *P. acuta*, | 1 | 45 | 0 | 0 | 0 |
| Limoges, | 2 | 52 | 0 | 0 | 0 |
| control | 4 | 78 | 0 | 0 | 0 |
| *Planorbis leucostoma*, | 1 | 26 | 0 | 0 | 0 |
| Saint-Léger-Magnazeix, | 2 | 44 | 0 | 0 | 0 |
| watercress bed | 4 | 57 | 0 | 0 | 0 |
| *P. leucostoma*, | 1 | 34 | 0 | 0 | 0 |
| Tersannes, | 2 | 52 | 0 | 0 | 0 |
| control | 4 | 71 | 0 | 0 | 0 |

* Live sporocysts of *F. hepatica*.
† Presence of a sporocyst and a first-generation free redia.

Table IV. – Experimental infections of snails originating from 10 populations with *Fasciola hepatica* in relation with their shell height at miracidial exposure.

infection (calculated in relation to the number of surviving snails at day 30 p.e.), and the number of cercariae per snail and per trematode. A comparison test of experimental frequencies, a $Chi^2$ test, and an one-way analysis of variance were used to establish levels of significance.

## RESULTS

### NATURAL INFECTIONS OF SNAILS WITH *FASCIOLA HEPATICA*

Snails harbouring rediae and cercariae of *F. hepatica* (Table III) were found in two watercress beds inhabited by *L. ovata* or *P. leucostoma*. The prevalences of these natural infections were 1.4 % and 0.1 %, respectively. Some sporocysts of *F. hepatica* were also noted in the *P. acuta* from two other sites but the prevalences were less than 1 . No *Fasciola* infection was noted in the snails from the two other sites.

Other trematode species (data not shown) were found in these snails. Sporocysts of *H. cylindracea* and immature rediae of *P. daubneyi* were present in the body of seven *L. ovata* and two *P. leucostoma*, respectively. Four other trematode species (unidentified) were also noted in *L. ovata*, *L. stagnalis*, or *P. acuta*.

### EXPERIMENTAL INFECTIONS OF SNAILS WITH *FASCIOLA HEPATICA*

Table IV gives the results obtained with mono-exposed snails in relation to their shell height at miracidial exposure. In the six populations of *P. acuta* and *P. leucostoma*, no cercarial shedding was noted. Only some sporocysts and an immature redia of *F. hepatica* were detected in their cadavers, so that the prevalences of infections ranged from 1.3 to 8.0 %. In contrast, cercarial sheddings were noted in *L. ovata* and *L. stagnalis*. The prevalence of this infection was significantly higher ($P < 0.05$) in the *L. ovata* originating from the watercress bed than in the control population. A significant decrease of this prevalence ($P < 0.05$) in relation to the increase of the snail size at miracidial exposure could also be noted. Similar findings could be noted for the prevalence of *F. hepatica* infection in *L. stagnalis*. The mean number of cercariae shed by the *L. ovata* living in the watercress bed significantly increased ($F = 3.32$, $P < 0.05$) in relation to the snail size at miracidial exposure. In contrast, no significant variation between the mean numbers of cercariae shed by the 1-mm groups was noted, whatever the snail species and population.

Despite the increase of exposures in number (Table V), no cercarial shedding of *F. hepatica* was noted in the six physidid and planorbid populations. Sporocysts, with or without immature rediae, were

| Snail species and population | Number of exposure | Number of snails: At day 30 p.e. | Number of snails: With live sporocysts | Number of snails: With sporocysts and immature rediae | Prevalence of infection in % |
|---|---|---|---|---|---|
| *Physa acuta*, Magnac-Bourg | 2 | 45 | 3 | 0 | 6.5 |
| | 3 | 35 | 2 | 0 | 5.7 |
| | 4 | 24 | 4 | 1 | 20.8 |
| *P. acuta*, Saint-Sornin-Leulac, | 2 | 57 | 1 | 0 | 1.7 |
| | 3 | 49 | 2 | 1 | 6.1 |
| | 4 | 41 | 4 | 2 | 14.6 |
| *P. acuta*, Thiat | 2 | 62 | 1 | 0 | 1.6 |
| | 3 | 51 | 1 | 1 | 3.9 |
| | 4 | 37 | 2 | 0 | 5.4 |
| *P. acuta*, control | 2 | 48 | 0 | 0 | 0 |
| | 3 | 31 | 0 | 0 | 0 |
| | 4 | 19 | 1 | 0 | 5.2 |
| *Planorbis leucostoma*, watercress bed | 2 | 28 | 1 | 0 | 3.5 |
| | 3 | 22 | 2 | 0 | 9.0 |
| | 4 | 12 | 4 | 0 | 33.3 |
| *P. leucostoma*, control | 2 | 31 | 0 | 0 | 0 |
| | 3 | 23 | 0 | 0 | 0 |
| | 4 | 18 | 0 | 0 | 0 |

Table V. – Experimental infections of 1-mm high physidid and planorbid snails with *Fasciola hepatica* in relation to the number of miracidial exposures (2, 3, or 4).

found in the cadavers of 12 *P. acuta* and seven *P. leucostoma*. The prevalences of infections increased with the number of exposures, as the number of surviving snails at day 30 p.e. inversely decreased. However, no significant difference between these prevalences was found, whatever the test used and the mode of comparison.

### Experimental infections of snails with an other trematode species and *Fasciola hepatica*

In single infections of *P. acuta* with *H. cylindracea* (Table VI), some sporocysts of this trematode were found in dissected snails but no significant difference between the prevalences of infections was noted. In dual infections of *P. acuta* with *H. cylindracea* and *F. hepatica*, some sporocysts of *F. hepatica* were noted in two snails. Negative results were obtained in single and dual infections of *P. leucostoma*.

In single infections of *P. leucostoma* with *P. daubneyi* (Table VII), sporocysts and first-generation rediae were found in three snails and no cercarial shedding occurred. In dual infections of this species with *P. daubneyi* and *F. hepatica*, five snails harboured larval forms of *P. daubneyi* or *F. hepatica*, and three shed low numbers of *F. hepatica* cercariae. One snail cadaver from this group was co-infected, with a single redia of *F. hepatica* and two rediae of *P. daubneyi*. Negative results were obtained in single and dual infections of *P. acuta*, except for the presence of *F. hepatica* sporocysts in two dually-exposed snails.

| Snail species and population | Parasite | Number of snails | | Prevalence of infection in % | Larvae of *H. cylindracea* |
|---|---|---|---|---|---|
| | | At day 30 p.e. | With larvae | | |
| *Physa acuta*, | *Haplometra cylindracea* only, | 62 | 1 | 1.6 | 2* |
| Magnac-Bourg | *H. cylindracea* + *F. hepatica* | 47 | 0 | 0 | – |
| Saint-Sornin-Leulac | *H. cylindracea*, | 71 | 2 | 1.6 | 2* |
| | *H. cylindracea* + *F. hepatica* | 55 | 1 (*F. hepatica*) | 0.8 | 1† |
| Thiat | *H. cylindracea*, | 61 | 0 | 0 | – |
| | *H. cylindracea* + *F. hepatica* | 52 | 1 (*F. hepatica*) | 1.9 | 1† |
| *Planorbis leucostoma* | *H. cylindracea*, | 32 | 0 | 0 | – |
| | *H. cylindracea* + *F. hepatica* | 11 | 0 | 0 | – |

* Second-generation sporocysts of *H. cylindracea*.
† First-generation sporocysts.

Table VI. – Experimental infections of 1-mm high physidid and planorbid snails with *Haplometra cylindracea* and/or *Fasciola hepatica*.

| Snail species and population | Parasite | Number of snails | | Prevalence of infection in % | Number of *Fasciola hepatica* cercariae (means ± S.D.) |
|---|---|---|---|---|---|
| | | At day 30 p.e. | With larvae | | |
| *Physa acuta*, | *Paramphistomum daubneyi* only, | 66 | 0 | 0 | 0 |
| Magnac-Bourg | *P. daubneyi* + *F. hepatica* | 54 | 0 | 0 | 0 |
| Saint-Sornin-Leulac | *P. daubneyi*, | 78 | 0 | 0 | 0 |
| | *P. daubneyi* + *F. hepatica* | 59 | 1* | 1.6 | 0 |
| Thiat | *P. daubneyi*, | 66 | 0 | 0 | 0 |
| | *P. daubneyi* + *F. hepatica* | 39 | 1* | 2.5 | 0 |
| *Planorbis leucostoma* | *P. daubneyi*, | 45 | 3† | 6.6 | 0 |
| watercress bed | *P. daubneyi* + *F. hepatica* | 34 | 5* | 14.7 | 3.5 ± 2.5 (3 snails) |

* Sporocysts (*P. acuta*) or rediae and cercariae (*P. leucostoma*) of *F. hepatica*.
† Sporocysts and first-generation rediae of *P. daubneyi*.

Table VII. Experimental infections of 1-mm high physidid and planorbid snails with *Paramphistomum daubneyi* and/or *Fasciola hepatica*.

## DISCUSSION

If the mean speed of water current (until 60 cm/sec on December) in the six watercress beds did not provide suitable conditions for the establishment of *L. truncatula* populations, it nevertheless allowed the colonization of the springs and ditch studied by more tolerant pulmonate species and natural infections of snails with *F. hepatica* could naturally occur in these places. Our investigations in these sites demonstrated the presence of larval forms in *L. ovata* and *P. leucostoma*, even though the prevalences of infections were low. Experimental infections with *F. hepatica* confirmed the role of both aforementioned species and indicated that the juvenile *L. stagnalis* could also sustain trematode larval development. These findings confirmed the results already reported by Kendall (1950) for both *Lymnaea* species, and by Abrous *et al.* (2000) for *P. leucostoma*. As the *Lymnaea* snails from beds could be infected at a greater size (until 4 mm for *L. ovata*) and had higher prevalences of infections than control snails, one might wonder whether these better performances in the former snails would not be the result of very local adaptations between parasite and both *Lymnaea*. An argument in support of this hypothesis was the report by Rondelaud (1993). According to this author, the populations of *L. truncatula* living in swampy meadows in central France and, consequently, having frequent natural encounters with *F. hepatica* showed higher survival rates, higher prevalences of infections, and greater cercarial productions than snail populations living on river banks and having scarce natural encounters with this trematode.

Despite mono- or bispecific infections, the results obtained with the four populations of *P. acuta* were not successful. A few sporocysts and immature rediae of *F. hepatica* were only found in some snails. In view of these results, it was difficult to comment the occurrence of human fasciolosis in the watercress beds in which *P. acuta* was the single freshwater species. As this snail was known to be invasive (Brackenburry & Appleton, 1993, for example) and could colonize open drainage ditches on siliceous soil (Vareille *et al.*, 1996), it might be hypothesized that these watercress beds would be late colonized by *P. acuta*, inducing the elimination of the snail host, probably *L. truncatula*, as this last species was very susceptible to a competition performed by an other freshwater snail species (Økland, 1990).

Monospecific infections of *P. leucostoma* demonstrated that this species was unable to sustain the larval development of *F. hepatica*. In contrast, the co-infection of this snail resulted in the shedding of some *F. hepatica* cercariae. These findings agreed with the data reported by Abrous *et al.* (1998) for *P. leucostoma* when this snail was subjected to successive exposures using *P. daubneyi* and *F. hepatica* miracidia. The presence of some immature rediae of *P. daubneyi* in the *P. leucostoma* living in this watercress bed may explain the case of human fasciolosis and suggests that a snail co-infection with both trematodes had occurred when the metacercariae of *F. hepatica* were present on the watercress eaten by man.

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