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▶ **To cite this version:**

Gilles Dreyfuss, Daniel Rondelaud. Fasciola gigantica and F hepatica: a comparative study of some characteristics of Fasciola infection in Lymnaea truncatula infected by either of the two trematodes. Veterinary Research, 1997, 28 (2), pp.123-130. hal-00902465

HAL Id: hal-00902465
https://hal.science/hal-00902465

Submitted on 11 May 2020

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Vet Res (1997) 28, 123-130
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Original article

Fasciola gigantica and *F hepatica*: a comparative study of some characteristics of *Fasciola* infection in *Lymnaea truncatula* infected by either of the two trematodes

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(Received 3 June 1996; accepted 10 September 1996)

Summary — Experimental infections were carried out using three *Lymnaea truncatula* populations and two *Fasciola* species in order to determine the trematode influence on six parameters of snail infection by either of the two trematodes. All experiments were performed using snails 4 mm long, two miracidia for each *L truncatula*, and a constant temperature of 20 °C. No significant influence of trematode species was detected in the following parameters: snail survival at day 30, the life span of infected snails (from miracidial exposure to snail death), the increase in shell length throughout the experiment, the duration of the patent period and the number of cercariae shed by infected snails. The frequency of cercaria-shedding snails was closely correlated with the particular *L truncatula* population and trematode species. A similar finding in two populations was also noted for the frequencies found in the infected snails who died without emission. The prepatent period had a longer duration in the *Fasciola gigantica*-infected groups. The percentages of floating cysts were greater in the *Fasciola gigantica* groups than in the *Fasciola hepatica* groups.

cercaria / metacercaria / *Fasciola* sp / *Lymnaea truncatula*

Résumé — *Fasciola gigantica* et *F hepatica* : étude comparative de plusieurs caractéristiques de l'infestation fasciolienne chez trois populations de *Lymnaea truncatula* infestées par l'un ou l'autre des trématodes. Des infestations expérimentales ont été réalisées

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chez trois populations de *Lymnaea truncatula* afin de déterminer l'influence du trématode sur les caractéristiques de l'infestation lorsque la même souche est parasitée par *Fasciola gigantica* ou par *F hepatica*. Toutes les expériences ont été effectuées en utilisant des mollusques de 4 mm de longueur, deux miracidiums pour chaque *L truncatula* et une température constante de 20 °C pour l'élevage des limnées. L'espèce du trématode n'a pas eu d'effet sur la survie des limnées au 30e jour, la durée de vie des mollusques infestés (depuis l'exposition aux miracidiums jusqu'à la mort des mollusques), l'accroissement de la longueur de la coquille au cours de l'expérience, la durée de la période patente et le nombre total de cercaires produites par les limnées infestées. Les fréquences des mollusques avec des émissions cercariennes ont été étroitement corrélées à la souche de *L truncatula* et à l'espèce du trématode utilisées pour ces expériences. Il en a été de même pour les pourcentages relevés dans deux populations chez les mollusques infestés qui sont morts sans émission. Des durées plus longues ont été observées dans les trois séries infestées par *F gigantica* pour la période prépatente. Les pourcentages des kystes flottants étaient plus importants dans le cas de *F gigantica* que dans le cas de *F hepatica*.

cercaire / métacercaire / *Fasciola* sp / *Lymnaea truncatula*

INTRODUCTION

Species of the *Lymnaeidae* family are known for their role as intermediate hosts in the life cycle of *Fasciola gigantica* or *Fasciola hepatica*. Work by Boray (1978) reviewed their aptitude for the development of these trematode's larval forms. Most reports, however, used a single snail-parasite system (Euzeby, 1971). Studies on the same population of lymnaeid snails infected by either of two trematodes are rare. Dreyfuss and Rondelaud (1995) demonstrated that parasite productivity was clearly higher in *Lymnaea tomentosa* infected by *F gigantica* than in the same snail population infected by *F hepatica*, while the number of miracidia per snail and the experimental conditions remained identical. It is therefore possible to suggest that the greater productivity of *F gigantica* rediae noted in *L tomentosa* ought to have an impact on the principal characteristics of snail infection such as survival rate or life span of infected snails. A logical question to pose is whether the parameters of snail infection are the same when the same population of *Lymnaea* is infected by either of the two *Fasciola*. To answer this question, we performed experimental infections using *Lymnaea truncatula* which was known to be a natural intermediate host of *F hepatica* (Boray, 1978) and a potential intermediate host of *F gigantica* under laboratory conditions (Rakotondravao and Rondelaud, 1991). Snails originating from three populations of *L truncatula* were exposed to miracidia of *F gigantica* or *F hepatica*, and six parameters of snail infection were studied.

MATERIALS AND METHODS

Experimental protocol

Three French populations of *L truncatula* living either in the department of Indre (Les Doucets, commune of Saint-Marcel for population A), and in the department of Haute-Vienne (Les Châtaigniers, commune of Peyrat-de-Bellac for colony B; Le Treuil, commune of Limoges-Landouge for population C) were used in the experiment. Colony A originated from a road ditch on calcareous soil, whereas populations B and C lived in swampy meadows situated on siliceous soil. These three populations were known to be devoid of natural trematode infection because regular sampling of snails had occurred these sites over 2 years and no trematode larval form was found in dissected snails. The snails were transported to the laboratory

under isothermal conditions and placed in standard breeding aquaria for an acclimatization period of at least 48 h at 20 °C. All experiments were performed using snail 4 mm long; this length corresponds to the preadult size for *L truncatula*. The *F gigantica* eggs were collected at the slaughterhouse of Tananarive (Madagascar), whereas those of *F hepatica* came from the slaughterhouse of Limoges (France). They were collected from the gallbladders of cattle with chronic *Fasciola* infections and were incubated for 20 days at 20 °C in complete darkness (Ollerenshaw, 1971; Rakotondravao, 1984).

Table I gives the characteristics of the six experimental groups. They were raised under constant conditions at 20 °C, with artificial lighting for 12 h (0700 to 1900 hours) at an intensity of 3 000 lux at the aquaria surface. Each *L truncatula* was exposed to two miracidia for 4 h. The choice of two miracidia per snail was preferred to that of one, three or more miracidia/snail because the infection rate was optimal without large mortality. The snails were subsequently raised until day 30 in closed-circuit aquaria in an air-conditioned room at 20 °C (five snails per litre of water). Control groups of uninfected snails were also formed (25 animals for each population). At day 30, the surviving snails from the control and experimental groups were isolated in 35 mm diameter petri dishes with 2 or 3 mL of water and a piece of lettuce. The recipients were placed in the same air-conditioned room at 20 °C. Every 2 days until the snail's death, a metacercarial count was performed and the water in the dish was changed. Non-cercarial shedding snails were dissected just after their death to detect the presence of *F hepatica* larval forms and to recognize uninfected snails from infected snails that died without shedding.

Parameters utilized

Survival rate on day 30 was calculated using the ratio between the number of survivors counted on this day and the number of snails at the onset of experiment. The frequency of snails with cercarial shedding (first category) was calculated in relation to the number of surviving snails on day 30. A similar protocol was used for the frequency of infected snails that died without emission (second category). The differences between the percentages were analyzed using a comparison test of experimental frequencies (Stat-Itcf, 1988).

The life span of the control and infected snails corresponded to the time interval between the onset of experiment or miracidial exposure, and the snail's death. The increase in the shell length throughout experiment corresponded to the difference between the value measured at miracidial exposure and that determined at snail's death. The duration of the prepatent period extended from miracidial exposure to the first day of cercarial shedding, whereas the patent period concerned the whole shedding period until the snail's death. The total number of metacercariae included all types (floating cysts, fixed cysts

Table I. Survival rate of infected snails on day 30 (each snail was exposed to 2 miracidia).

	L truncatula *population. Number (%) of survivors on day 30*		
	A	*B*	*C*
F gigantica	165 (55)	146 (49.3)	241 (54.2)
F hepatica	155 (51.6)	140 (46.6)	178 (59.3)

The number of snails at the onset of experiment was 300, except for *L truncatula* population C infected with *F gigantica* ($n = 444$).

and free cysts). Mean values were determined from the individual percentages of each parameter and standard deviations were calculated. These values were then analyzed by a one-way analysis of variance (Stat-Itcf, 1988).

RESULTS

Table I gives the survival rates on day 30 in the experimental groups. The trematode species had no significant influence on the survival time of snails in these groups. No dead snails were observed among the 25 snails of control groups in the populations A and C. In the colony B control group, the survival rate was 96% (24 snails) on day 30.

Table II indicates the number of snails in each category and their frequency of infection compared to the number of surviving snails on day 30. The percentage of snails with cercarial shedding varied with trematode species and ranged from 15 to 19% in the *F gigantica*-infected groups and from 47 to 51% in the *F hepatica*-infected groups. Infected snails that died without emission were observed and their percentages ranged from 11 to 18% in the *F gigantica*-infected groups, from 14 to 37% in the *F hepatica*-infected groups. The trematode species had a significant influence on the frequency of infected snails with cercarial shedding ($P < 0.001$), and on that of infected snails that died without emission in populations A and B ($P < 0.001$).

Table III groups the results obtained from the control and infected snails with or without cercarial shedding. Differences in the length of survival time according to snail category were noted. Survival times were shorter in each group of infected snails than in controls. They were also reduced in the snails without emission (category 2). Significant differences between the mean values of controls and those of infected snails were noted for each population separately considered ($P < 0.001$ in the six groups), whereas the population and trematode species had no significant influence.

Table III gives also the mean values found for the increase in shell length throughout the experiment. No significant difference in the increase was noted between the three populations of *L truncatula* whatever the experimental group and the category of snails.

Table IV indicates the lengths of the prepatent period and those of the patent period. Significant differences in the mean

Table II. Frequency of snails infected by category in the six groups.

	Frequency of snails per category (%)		
	Non-infected	*Infected without shedding*	*Infected with shedding*
Population A			
F gigantica	71	13.9	15.1
F hepatica	19.3	27.2	53.5
Population B			
F gigantica	70.5	11.1	18.4
F hepatica	15.7	37.2	47.1
Population C			
F gigantica	62.7	17.8	19.5
F hepatica	30.3	15.2	54.5

duration of the prepatent period between the *F gigantica* groups and *F hepatica*-infected snails were noted for the three populations ($P < 0.001$). Differences in the mean lengths of the patent period between the three populations were not significant.

Table V groups the numbers of cercariae shed per snail in the six experimental groups. Mean values ranged from 135 and 174 cercariae in the *F gigantica*-infected groups, and from 121 to 209 in those with *F hepatica*. The trematode species had no significant influence on these results. Some cercariae died after their exit from the snail but their number was very low (0.06% in the six groups). Other cercariae fixed on a support, or formed free metacercariae on the bottom of the recipients, or turned into floating cysts. In the *F gigantica*-infected groups, the percentage of floating cysts ranged from 17.1 to 32.7%. In the groups with *F hepatica*, the percentage did not exceed 10%.

DISCUSSION

Many factors can affect intramolluscan-trematode dynamics. They include a variety of forces, both internal and external to the snail, and affect the manner in which the larval development occurs (Esch and Fernandez, 1994). Even though the interactions between the snail and the trematode depends on the snail's suitability for the parasite and perhaps on the frequency of natural encounters between the two partners in the field (Rondelaud, 1993), it was interesting to determine trematode influence on the parameters of snail infection when the snail species can assure the complete larval development of two trematodes originating from the same family.

The survival rates found in the three populations of *L truncatula* agree with the percentages reported by several authors in other French populations of *L truncatula* when they used two miracidia of *Fasciola* sp per snail

Table III. Survival time of infected snails by category and increase in shell length in the six groups.

	Survival time in days[a] *(number of snails)*		*Increase*[b] *in shell length (mm)*[c] *(number of snails)*	
	Infected without shedding	*Infected with shedding*	*Infected without shedding*	*Infected with shedding*
Population A				
F gigantica	62.3 ± 9.6 (23)	73.4 ± 13 (25)	2.4 ± 1.1 (23)	3.1 ± 1.1 (25)
F hepatica	56.7 ± 11.3 (42)	67.4 ± 13.2 (83)	2.5 ± 0.9 (42)	2.81 ± 1.2 (83)
Population B				
F gigantica	67.4 ± 14.9 (16)	77.2 ± 19.1 (27)	2.1 ± 1.3 (16)	2.4 ± 1.4 (27)
F hepatica	61.3 ± 13.2 (52)	74.1 ± 14.1 (66)	2.3 ± 1.5 (52)	2.5 ± 1.2 (66)
Population C				
F gigantica	51.3 ± 31 (43)	78.2 ± 32.6 (47)	2.1 ± 1 (43)	2.7 ± 1.3 (47)
F hepatica	57.2 ± 20.7 (27)	84.7 ± 25.1 (97)	1.8 ± 1.2 (27)	2.1 ± 1.7 (97)

[a] In the control group, survival time was 97.6 ± 13.8 days (population A), 107.3 ± 17.4 days (population B), and 112.3 ± 21.6 days (population C); [b] The shell length of snails was 4 mm at the time of miracidial exposure. [c] In the control snails, the increases in shell lengths were 2.9 ± 1 mm (population A), 2.9 ± 1.2 mm (population B), and 3.2 ± 1.2 mm (population C).

(Audousset et al, 1989; Rakotondravao and Rondelaud, 1991; Dreyfuss and Rondelaud, 1994a). Our results demonstrated that the trematode species had no significant influence on the survival of *L truncatula* on day 30 when miracidial exposure was performed using snails 4 mm long. However, this finding concerned only snails individually infected with two miracidia. Indeed, Rondelaud and Barthe (1982) found a decrease in the snail survival rate on day 30 when the number of miracidia used for each snail increased (from 27 to 82% in snails each infected by 1 to 20 *F hepatica* miracidia, respectively).

The frequency of snails with cercarial shedding ranged widely and this variability must be attributed to the particular population of snail and trematode species (Boray, 1966). In

Table IV. Durations of the prepatent period and patent period in infected snails.

L truncatula	*Number of snails*	*Duration of the prepatent period (days)*	*Duration of the patent period (days)*
Population A			
F gigantica	25	59.2 ± 8.3	17.3 ± 11.7
F hepatica	83	41.9 ± 5.0	23.8 ± 14.3
Population B			
F gigantica	27	57.6 ± 7.7	21.1 ± 12.3
F hepatica	66	42.7 ± 6.4	25.6 ± 16.7
Population C			
F gigantica	47	61.2 ± 10.5	23.8 ± 13.1
F hepatica	97	47.2 ± 6.7	28.3 ± 16.2

Mean values are given with their standard deviations.

Table V. Number of cercariae shed per snail and their outcome.

L truncatula	*Number of cercariae (number of snails)*	*Outcome of cercariae*			
		Dead cercariae	*Fixed cysts*	*Floating cysts*	*Free cysts*
Population A					
F gigantica	135.1 ± 113.8 (25)	1	2 797	578	1
F hepatica	121.2 ± 98.3 (83)	7	9 640	412	–
Population B					
F gigantica	157.2 ± 132.1 (27)	3	2 991	1 243	7
F hepatica	127.2 ± 109.3 (66)	8	7 783	604	–
Population C					
F gigantica	174.3 ± 104.5 (47)	5	5 485	2 669	3
F hepatica	209.4 ± 187.6 (97)	11	19 265	1 035	–

Mean values are given with their standard deviations.

contrast, some infected snails died without shedding in all experimental groups and this result was more difficult to interpret. The hypothesis propounded by Kendall (1950) that the absence of cercarial shedding from *F hepatica*-infected *L glabra* and *L palustris* was due to the snail's anatomical configuration cannot be retained in the context of this study. The most valid assumption to explain this absence of shedding would be the tissue lesions which appeared in the snail after miracidium penetration and developed over some weeks until snail death (Sindou et al, 1991a,b). Under these conditions, the physiological state of the intermediate host would not permit cercaria shedding.

The literature does not give any quantitative data on the survival time of infected snails. Our results demonstrated greater values for snails manifesting cercarial shedding and can only be explained by assuming the probable existence of a selection; the absence of emission would induce snail's death after a certain time, under the influence of factors whose nature must be determined. The presence of shedding would result in greater snail survival, thus suggesting a probable selection in the infected snails in relation to their physiological state.

Longer durations found for the prepatent period in *F gigantica*-infected snails agree with the figures furnished by Rakotondravao and Rondelaud (1991) for *L truncatula*, Da Costa et al (1994) for *Lymnaea natalensis*, and Dreyfuss and Rondelaud (1994b) for *L tomentosa* (between days 52 and 58 at 23 °C). In *F hepatica*-infected snails, the onset of the patent period (on days 42 or 49 on average) agrees with the figures reported by Boray (1969), and Rondelaud and Barthe (1982, 1987), if the maintenance temperature is taken into account. From this discordance, it can be suggested that the development of *F gigantica* larvae needed more time than those of *F hepatica* and this finding must be related to the higher productivity found for the former trematode (Dreyfuss and Rondelaud, 1995). In contrast, the durations of the patent period found in *F gigantica*- and *F hepatica*-infected groups were similar, indicating the absence of trematode influence on this parameter of snail infection.

Higher percentages of floating cysts were recorded from *F gigantica*-infected snails and suggested that they were trematode dependent. However, the species of the snail used for the experimental infection had also an influence on these percentages, which were 26.7% in *L natalensis* (Da Costa et al, 1994) and 29.3% in *L tomentosa* (Dreyfuss and Rondelaud, 1994b) when these snails were infected by *F hepatica* under laboratory conditions. Thus, the numbers of these floating cysts depended on the particular trematode and snail species.

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