FASCIOLA HEPATICA : THE INFLUENCE OF THE DEFINITIVE HOST ON THE CHARACTERISTICS OF INFECTION IN THE SNAIL *LYMNAEA TRUNCATULA*

RONDELAUD D.* & DREYFUSS G.**

Summary :

Investigations were carried out in *Lymnaea truncatula* to determine whether redial burden and shedding of *F. hepatica* cercariae vary when the same population of *Lymnaea truncatula* is exposed to miracidia that hatched from eggs collected in different naturally infected definitive hosts (cattle, sheep, and rabbits originating from the same region). Snails measuring 4 mm in height were each exposed to two miracidia hatched from these eggs, and were then raised at 20 °C for 30 days. The prevalence of infected snails was 35 %, 66 %, and 86 % in the groups exposed to miracidia originating from rabbits, sheep, and cattle respectively. Cercariae were shed from snails of the three groups, however, shedding varied greatly depending on the origin of the miracidia. The total number of cercariae was significantly decreased in the group infected with miracidia from rabbits than in the others (54.5 per snail vs 157.8 and 216.4). The circadian rhythm in the shedding of cercariae was also changed in the former group, with maximal shedding between 4 a.m. and 8 a.m. No significant difference was noted in the other characteristics of snail infection between the three groups. Histological examination of infected snails revealed that the total number of rediae was significantly decreased at day 30 in the snails infected from miracidia that originated from rabbits than in the other groups (a mean of 21.3 vs 38.4 and 43.7). Degenerated, free rediae were most numerous in the former group than in the two others. The definitive host species may play a role in the development of *Fasciola* infection in the snail by limiting redial and cercarial burdens. From this study, trematode eggs collected in cattle or sheep are more efficient for transmission of the disease than those obtained from rabbits.

KEY WORDS : cercaria. definitive host. *Fasciola hepatica*. *Lymnaea truncatula*. parasite productivity. redia.

Résumé : *FASCIOLA HEPATICA* : L'INFLUENCE DE L'HÔTE DÉFINITIF SUR LES CARACTÉRISTIQUES DE L'INFESTATION CHEZ LE MOLLUSQUE *LYMNAEA TRUNCATULA*

Des observations ont été réalisées chez Lymnaea truncatula *afin de déterminer l'existence de modifications dans la charge rédienne et les émissions cercariennes lorsque la même population de limnées est exposée à des miracidiums qui proviennent d'œufs récoltés chez des bovins, des ovins ou des lapins infestés naturellement, vivant sur les mêmes exploitations. Des limnées hautes de 4 mm ont été exposées chacune à deux miracidiums par mollusque avant d'être élevées à 20 °C pendant 30 jours. La prévalence de l'infestation est de 35, 66 et 86 % respectivement dans les groupes lapin, ovin et bovin par rapport au nombre de survivants au 30e jour. Des cercaires ont été émises par les mollusques des trois groupes mais le nombre total de cercaires est plus faible dans le groupe lapin que dans les deux autres (54,5 par mollusque au lieu de 157,8 et 216,4 respectivement). Le rythme circadien dans les émissions cercariennes est aussi changé dans le groupe lapin, avec des émissions maximales entre 4 et 8 heures. L'examen histologique de mollusques infestés montre que le nombre total de rédies est plus faible au 30e jour dans le groupe lapin que dans les deux autres groupes (21,3 par limnée au lieu de 38,4 et 43,7 respectivement). Les rédies indépendantes et dégénérées sont plus nombreuses dans le premier groupe. L'espèce de l'hôte définitif peut donc jouer un rôle dans le développement de l'infestation fasciolienne chez le mollusque en limitant le nombre des rédies et la production cercarienne. Il ressort de cette étude que les œufs du trématode récoltés chez les bovins ou les ovins sont plus efficaces pour la transmission de la maladie que ceux provenant de lapins.*

MOTS CLÉS : *cercaire.* Fasciola hepatica. *hôte définitif.* Lymnaea truncatula. *productivité parasitaire. rédie.*

INTRODUCTION

Fasciolosis is an economically important disease caused by *Fasciola hepatica* in temperate regions, and its occurrence is dependent on the presence of *Lymnaea truncatula* populations for the development of parthenitae. This trematode can affect a wide range of susceptible hosts including sheep, cattle, and also wild mammals such as rabbits (Haroun and Hillyer, 1986).

Little data are currently available on the bionomics of *Fasciola* infection in *L. truncatula* when the same colony of snails is exposed to different strains of *F. hepatica* miracidia. Differences in infection rate and cercaria production were reported by Boray (1966, 1969) in the same population of *L. truncatula* exposed to miracidia from different geographical origins. Because of this last finding the following questions arose : what is the success of intermediate host infection when different origins of *F. hepatica* are tested ? and, consequently, what is the best origin of trematode eggs for transmission of the disease ? To answer these questions, we subjected the same population of *L. truncatula* to miracidia that originated from eggs collected in cattle, sheep or wild rabbit gallbladders.

* Faculté de Médecine, Laboratoire d'Histopathologie Parasitaire and ** Faculté de Pharmacie, Laboratoire de Parasitologie, 2, rue du Docteur Raymond Marcland, F-87025 Limoges Cedex, France.
Correspondence : Dr. D. Rondelaud, Faculté de Médecine, F-87025 Limoges Cedex. Tel. : 55.43.58.33 – Fax : 55.43.58.01.

Investigations were carried out in two ways : a) the cercaria production of *F. hepatica* from these snails and b) the histological examination of the redial burden at day 30 of experiment.

MATERIALS AND METHODS

SNAILS AND PARASITE

The population of *L. truncatula* was living in a swampy meadow at Le Treuil, Commune of Limoges-Landouge, Department of Haute-Vienne (France). This colony was known to be devoid of any trematode infection because of regular sampling at the site and histological examination in 70 % of the snails collected. A total of 600 snails (height, 4 ± 0.1 mm) were collected in this meadow. They were transported to the laboratory in isothermal conditions and acclimatized for at least 48 hours in standard aquaria before being subjected to the experiment.

The eggs of *F. hepatica* were collected at the slaughterhouse of Limoges (France) from gallbladders of naturally infected cattle or sheep whose liver was distrained for distomatosis. The two oxen originated from a herd which was living in a first farm in the Commune of Nieul, Department of Haute-Vienne, and the three sheep came from another farm located in the same valley, near the first farm. A total of seven wild rabbits (*Oryctolagus cuniculus*) were killed by hunters in this valley and contained two to five adult parasites in their liver. Eggs were also collected from gallbladders within two hours after the death of the rabbits. All eggs were incubated for 20 days at 20° C in total darkness according to the method described by Ollerenshaw (1971).

EXPERIMENTAL PROTOCOL

Three groups of 200 snails each were constituted. The snails of the first group were individually exposed for four hours in 35-mm petri dishes to two *F. hepatica* miracidia that had hatched from eggs collected in cattle. The same protocol of infection was used in the two other groups but the *Fasciola* eggs came from sheep or from rabbit. The snails were then raised in closed-circuit aquaria for 30 days at 20 °C, with five snails per liter of water. Half of the water volume (calcium content, 12 mg/l) contained in aquaria was changed twice weekly.

At day 30, the surviving snails of each group were divided in two subgroups. The snails from the first subgroup were individually placed in 35-mm diameter petri dishes with 2-3 ml of water and a piece of lettuce. Daily monitoring consisted of counting metacercariae and changing water until the snail's death. The shell height of the dead snails was then measured. The snails from the second subgroup were dipped at day 30 into Bouin's fixative, following by immediate breaking of the shell under the stereomicroscope. Serial sections, 5 µm thick, were cut and stained using Harris' hematoxylin-modified Gabe's trichrome stains.

A special count was performed every hour for three days in infected snails of the first subgroups (10 per group) to determine the time of maximal shedding.

PARAMETERS UTILIZED

Snail infection rates were calculated by dividing the number of infected snails by the number of survivors on day 30.

The parameters of cercarial sheddings were the number of snails that shed cercariae, the onset and the duration of the patent period, the post-mortem shell height, the total number of metacercariae, the percentage of floating cysts (calculated in relation to the total number of metacercariae), the number of shedding waves, the number of snails that died after the first wave, and the duration of this first wave. Lastly, we also considered the different time periods (of one hour each) for which cercaria production was maximal.

Quantification of the total number of rediae, dependent rediae, degenerated free rediae (recognized by flattened, often triangular and pycnotic nuclei of morulae or procercarial embryos), and live free rediae was performed at day 30 by histological examination of the serial sections. Redial maturity was determined by the presence of cercariae and procercariae, and was expressed as a percentage in relation to the total number of live, independent rediae.

Mean values and standard deviations were determined in each group for the different parameters. They were also calculated for the number of cercariae recorded in the time periods. The mean values were subjected to Anova or to the comparison test of experimental frequencies (Stat-Itcf, 1987).

RESULTS

At day 30, the number of surviving snails was respectively 170, 156, and 108 in the cattle, sheep, or rabbit infected groups (out of 200 snails per group initially exposed to miracidia). The number of snails with live parthenitae on serial sections was respectively 62, 55, and 17 whereas in infected snails with or without emission, it was 84, 48, and 21. The overall prevalence of *Fasciola* infection in these groups was also significantly higher ($p < 0.01$) in snails infected with miracidia of cattle and sheep origins (86 % and 66 % respectively) than in those infected with miracidia of rabbit origin (35 %).

CERCARIAL SHEDDING

Table I gives the different results recorded in the infections derived from the three origins of *Fasciola* eggs. The frequency of snails with shedding was 80.9% in the cattle group, 64.5% in the sheep group, and 47.6% in the rabbit group. The onset of the patent period was similar in the three groups (from day 45 to day 51). The mean duration of this period ranged from 35 days (in the rabbit group) to 60 days in the cattle group but the differences in the mean numbers between these three groups were insignificant. The shell height was also similar in the three groups (a mean of 6 to 6.4 mm).

Cercaria production was significantly higher ($F = 31.2$; $p < 0.001$) in the cattle and sheep groups than in the rabbit group (216 and 157 respectively per snail instead of 54). The percentage of floating cysts was almost similar in the three groups (from 5.1 to 6.4 %). Shedding waves were more numerous in the cattle and sheep groups (9 and 8 respectively) than in the rabbit group (3 waves). Eighty percent of the snails died after the first shedding wave in the rabbit group whereas only 23.5% died in the cattle group and 45.1 % in the sheep group. Lastly, there were no significant differences in the mean duration of the first wave between the three groups.

Figure 1 gives the distribution of the number of cercariae in relation to the different time periods during the day. Sheddings were maximal between midnight and 1 a.m., and between midnight and 2 a.m. respectively in the sheep and cattle groups. In the rabbit group, the period of maximal shedding was different, occurring from 4 to 8 a.m.

REDIAL BURDEN AT DAY 30

The total number of rediae was 21.3 ± 10.7 in the rabbit group, 38.4 ± 8.4 in the sheep group, and 43.7 ± 10.9 in the cattle group. A significant difference in the mean values between the rabbit group and the two others was noted ($F = 23.1$; $p < 0.001$); it was not significant between the two latter groups.

Figure 2 demonstrates that degenerated, free rediae were more numerous in the rabbit group than in the sheep and cattle groups (a mean of 12.7 instead of 8.6 and 9.9 respectively). A significant difference in the mean values between the rabbit group and those of the other two groups was noted ($F = 11.03$; $p <$

Groups	Miracidia of *F. hepatica*		
	Cattle origin	Sheep origin	Rabbit origin
Number of snails: - with live parthenitae, - with shedding, - without shedding. Frequency of snails with shedding	 84 68 14 80.9 %	 48 31 17 64.5%	 21 10 11 47.6%
Patent period : - onset (days) - duration (days)	 51.2 ± 4.6 60.4 ± 23.7	 50.3 ± 7.2 47.3 ± 22.4	 45.4 ± 2.4 30.2 ± 13.3
Shell height at snail's death (mm)	6.4 ± 1.7	6.1 ± 1.4	6.0 ± 2.3
N° of metacercariae derived from 200 initial snails per origin	216.4 + 52.3	157.8 ± 41.5	54.5 ± 15.8
Percentage of floating cysts	6.4 ± 1.3 %	5.6 ± 2.3 %	5.1 ± 3.7 %
Number of shedding waves	9	8	3
Number of snails that died after the first wave	16	14	8
Duration of the first wave (days)	3.4 ± 1.6	2.7 ± 2.1	3.1 ± 3.2

Table 1. – The general characteristics of cercarial sheddings in the snail groups infected with miracidia of different origins.

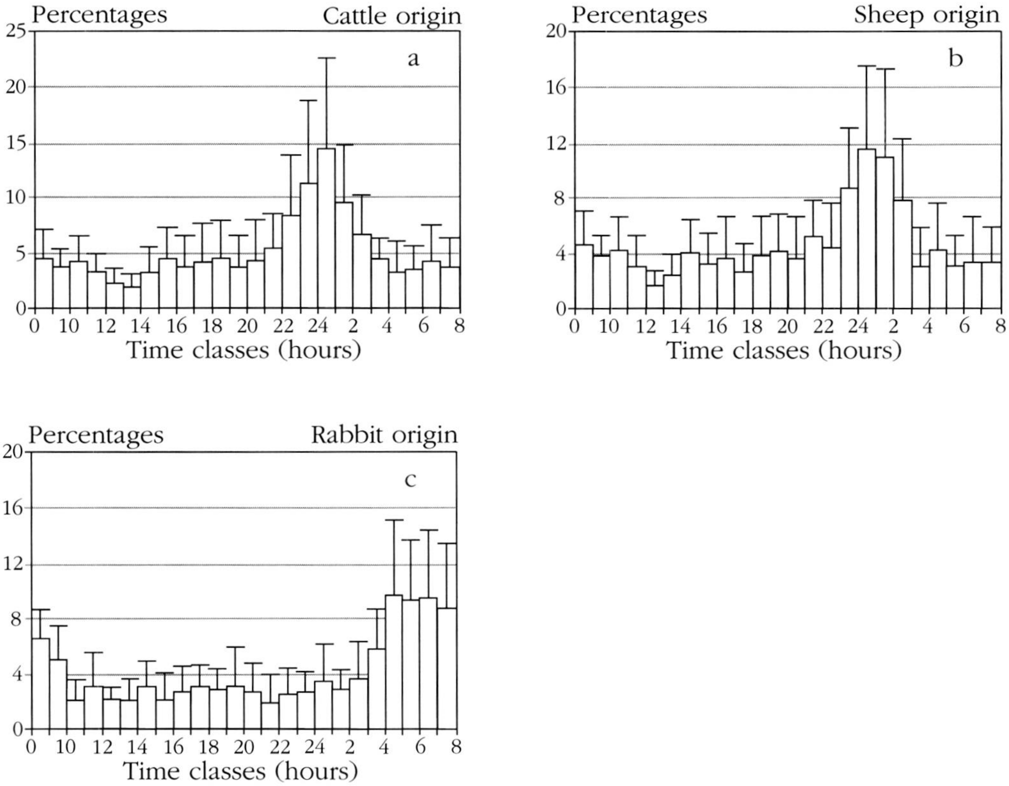

Fig. 1. – Numerical distribution of cercariae shed in *L. truncatula* infected with miracidia of three origins (cattle, sheep, or rabbit) during the day.

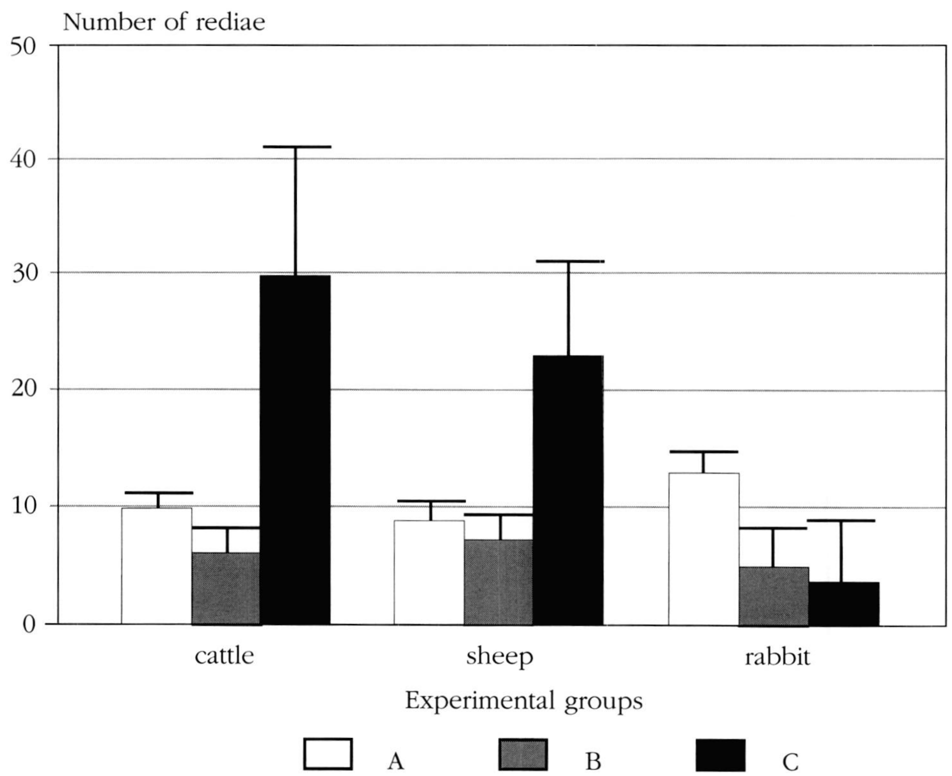

Fig. 2. – Numerical distribution of *F. hepatica* rediae in *L. truncatula* infected with miracidia of three origins. Abbreviations : A (degenerated, free rediae). B (dependent rediae). C (live, free rediae).

0.01) but the difference between the sheep and the cattle groups was not significant.

Live rediae were diversely affected. No significant differences in the mean numbers between the three groups were noted for dependent rediae (4.9 to 7.3 per snail). On the other hand, the number of free rediae was 3.7 in the rabbit group instead of 22.7 in the sheep group and 29.8 in the cattle group. Comparison of the mean values revealed a significant difference only between the rabbit group and the two others ($F = 21.47$; $p < 0.001$).

Mature rediae represented 44 to 52 % of live, free rediae at day 30 in the cattle and sheep groups. In the rabbit group, all these latter rediae were mature at this date (data not shown).

DISCUSSION

From our results, the characteristics of *Fasciola* infection in the same population of *L. truncatula* vary when the miracidia originate from eggs collected from different definitive hosts. These modifications principally involved snails that were exposed to miracidia that originated from eggs collected in rabbits. These findings indicate that the definitive host species plays a role in the development of *Fasciola* infection in the snail by limiting the number of rediae and the cercaria production. From these results, it shoud also be noted that *Fasciola* eggs collected in cattle and sheep are more efficient for transmission of the disease than those obtained from rabbit.

Problems of compatibility between snail strain and *F. hepatica* have already been studied by Boray (1969, 1978) who showed that prevalence and cercaria production can be modified according to the strain's origin. Some variation in the prevalence of *F. hepatica* (41 % to 51 % at day 30 of experiment) and the total number of rediae (18 to 22 per snail) has also been noted by Rondelaud and Barthe (1982) in the same colony of *L. truncatula* exposed to different strains of miracidia that originated from cattle from three geographical origins.

These results demonstrate that the definitive host plays a greater than previously believed role in the development of *Fasciola* parthenitae in the snail. Hillyer and Haroun (1986) demonstrated in their review that the development of adult trematode in the definitive host varies in relation with the mammalian species and, in this context, it is logical to assume that the trematode eggs were directly affected by the developmental conditions of the adult parasite. These changes in several rabbit group parameters concord with Euzeby's observations (1971) when he discovered that the hatching rate of *F. hepatica* eggs excreted by rabbits was only 30 %, whereas it ranged from 60 to 85 % from the eggs collected in cattle. According to Euzeby (1971), growth of the adult parasite in the rabbit is impaired, diminishing egg fertility. This hypothesis provides an explanation for the reduction in *Fasciola* prevalence, and the number of rediae and cercariae in the rabbit group.

On the other hand, two results warrant particular comments. First, the significant increase recorded in the number of degenerated, free rediae in the rabbit group is difficult to interpret in the light of our current knowledge on the *L. truncatula-F. hepatica* model. If the developmental pattern of *F. hepatica* rediae proposed by Rondelaud and Barthe (1978, 1982) is accepted, it is logical to assume that the development of *Fasciola* parthenitae in the snail body would be inhibited by the host during the first two weeks after exposure, and that this inhibition would diminish and even disappear in subsequent weeks. This process would be enhanced in parthenitae that hatched from eggs collected in rabbits as compared with those which originated from eggs collected in cattle and sheep. This hypothesis is based on the numbers of degenerated rediae and those of dependent rediae found in the three groups.

Second, the results pertaining to the circadian rhythm in cercarial sheddings found in the cattle and sheep groups concord with data reported by Audousset *et al.* (1989) in *L. truncatula* where maximal shedding occurred between midnight and 1 a.m. On the other hand, it is more difficult to explain why maximal sheddings were recorded between 4 and 8 a.m. in the rabbit group. The changes in circadian rhythm reported by Mouahid and Théron (1986), Pages and Théron (1990) in the cercarial sheddings of *Schistosoma* sp. using hybridization of different trematode species or of different strains for the same trematode species do not apply to our result in the rabbit group because the three strains of miracidia originated from definitive hosts from the two farms. The most likely hypothesis would be to assume that a disturbance in cercarial sheddings occurs whose nature has yet to be elucidated.

REFERENCES

AUDOUSSET J.C., RONDELAUD D., DREYFUSS G. & VAREILLE-MOREL C. Les émissions cercariennes de *Fasciola hepatica* L. chez le mollusque *Lymnaea truncatula*. Müller. À propos de quelques observations chronobiologiques. *Bulletin de la Société Française de Parasitologie*, 1989, *7*, 217-224.

BORAY J.C. Studies on the relative susceptibility of some lymneids to infection with *Fasciola hepatica* and *F. gigantica* and on the adaptation of *Fasciola* spp. *Annals of tropical Medicine and Parasitology*, 1966, *60*, 114-124.

BORAY J.C. Experimental fascioliasis in Australia. *Advances in Parasitology*, 1969, *7*, 95-210.

BORAY J.C. The potential impact of exotic *Lymnaea* spp. on fascioliasis in Australasia. *Veterinary Parasitology*, 1978, *4*, 127-141.

EUZEBY J. Les maladies vermineuses et leurs incidences sur la pathologie humaine. Tome II. Section 2. Book 1. Vigot frères ed., Paris, 1971, 798 p.

HAROUN E.M. & HILLYER G.V. Resistance to fascioliasis. A review. *Veterinary Parasitology*, 1986, *20*, 63-93.

MOUAHID A. & THÉRON A. *Schistosoma bovis* : variability of cercarial production as related to the snail hosts : *Bulinus truncatus*, *B. wrighti* and *Planorbarius metidjensis*. *International Journal for Parasitology*, 1986, *17*, 1431-1434.

OLLERENSHAW C.B. Some observations on the epidemiology of fascioliasis in relation to the timing of molluscicide applications in the control of the disease. *Veterinary Record*, 1971, *98*, 152-164.

PAGES J.R. & THÉRON A. Analysis and comparison of cercarial emergence rhythms of *Schistosoma haematobium*, *S. intercalatum* and *S. bovis*, and their hybrid progeny. *International Journal for Parasitology*, 1990, *20*, 193-197.

RONDELAUD D. & BARTHE D. Arguments et propositions pour une nouvelle interprétation de l'évolution de *Fasciola hepatica* L. dans *Lymnaea (Galba) truncatula* Müller. *Annales de Parasitologie humaine et comparée*, 1978, *53*, 201-213.

RONDELAUD D. & BARTHE D. Les générations rédiennes de *Fasciola hepatica* L. chez *Lymnaea truncatula* Müller. À propos des effets de plusieurs facteurs. *Annales de Parasitologie humaine et comparée*, 1982, *57*, 245-262.

STAT-ITCF, 1987. Manuel d'utilisation. Institut Technique des Céréales et des Fourrages, Service des études Statistiques, Boigneville, 210 p.

Accepté le 28 avril 1995