FASCIOLA HEPATICA : A STUDY OF THE SHEDDING OF CERCARIAE FROM *LYMNAEA TRUNCATULA* RAISED UNDER CONSTANT CONDITIONS OF TEMPERATURE AND PHOTOPERIOD

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Summary :

Investigations on the shedding of cercariae of *Fasciola hepatica* were carried out in *Lymnaea truncatula* in order to verify the existence of a low-frequency periodicity in the numerical distribution of metacercariae per snail and per day when the snails are raised under controlled conditions. Preadult *L. truncatula* were thus collected in the field, individually exposed to two miracidia, and subsequently raised until their death under constant temperature (20°C) and photoperiod (12 h/12 h diurnal rhythm). The 102 snails shedding parasites produced 24,325 metacercariae of which 5 % were floating cysts. The daily production of cercariae was maximal during the first 30 days of the patent period, subsequently decreased until day 114, and ceased on day 124. No infradian-type rhythm was noted in the daily distribution of mean values. The snails shed their cercariae in one to 14 waves; 20.6 % and 15.7 % of the snails produced their parasites in four and five periods respectively. The authors suggest that the seven-day periodicity found by Audousset *et al.* (1989) in the daily distribution of cercariae produced by three colonies of *L. truncatula* raised in seminatural conditions must be attributed only to the influence of environmental factors.

KEY WORDS : cercaria, metacercaria, cercarial shedding, *Fasciola hepatica*, *Lymnaea truncatula*.

MOTS CLES : cercaire, métacercaire, émission cercarienne, *Fasciola hepatica*, *Lymnaea truncatula*.

Résumé : *FASCIOLA HEPATICA* : ÉTUDE DES ÉMISSIONS CERCARIENNES CHEZ *LYMNAEA TRUNCALATA* ÉLEVÉE SOUS DES CONDITIONS CONSTANTES DE TEMPÉRATURE ET DE PHOTOPÉRIODE

Des observations sur les émissions cercariennes de *Fasciola hepatica* ont été réalisées chez *Lymnaea truncatula* afin de vérifier l'existence d'une périodicité avec une basse fréquence dans la distribution numérique des métacercaires par mollusque et par jour lorsque les limnées sont élevées sous des conditions contrôlées. Des mollusques préadultes ont été ainsi prélevés sur le terrain, exposés individuellement à deux miracidiums et maintenus par la suite jusqu'à leur mort sous des conditions constantes de température (20°C) et de photopériode (12 heures diurnes). Les 102 limnées ont fourni 24.325 métacercaires avec 5 % de kystes flottants. La production journalière est maximale dans les 30 premiers jours de la période patente et diminue ensuite jusqu'au 114^e jour pour cesser au 124^e jour. Il n'y a pas de rythme de type infradien dans la distribution journalière des moyennes. Les mollusques ont émis leurs parasites sur 1 à 14 vagues, avec 20,6 % et 15,7 % des limnées produisant leurs cercaires en quatre et cinq périodes. Les auteurs suggèrent que la périodicité de sept jours trouvée par Audousset *et al.* (1989) dans la distribution journalière des cercaires produites par trois colonies de *L. truncatula* élevées dans des conditions semi-naturelles doit être attribuée à l'influence des seuls facteurs du milieu.

Cercariae of *Fasciola hepatica* are shed from the host snail in one or several times during the patent period. Studies on this shedding have already been carried out in different *Lymnaea* species by several authors. Roberts (1950) found that the infected snails regularly shed their cercariae over a week and more whereas Krull (1933), Kendall and McCullough (1951), Bouix-Busson *et al.* (1985) reported that cercarial emission was discontinuous with shedding waves separated by periods of rest. Audousset *et al.* (1989) confirmed the existence of these shedding waves by studying three populations of infected *Lymnaea truncatula* that were subjected to variable daily temperatures ranging between 8° and 22° C. In addition, their observations demonstrated that shedding occurred with an infradian-type

rhythm with a periodicity of seven days. In view of these last results, the question arises whether the characteristics of shedding of cercariae are related to environmental changes (e.g. temperatures or photoperiod) or if they were due to internal rhythm in *L. truncatula*. To answer this question, we thus studied shedding in *L. truncatula* collected in the field and raised under constant conditions in order to assess the part of environmental determinism in shedding rhythms.

Preadult snails measuring 4 ± 0.2 mm in height were collected in May and June in two meadows at Le Treuil in the 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. 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 from

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Fig. 1. – Cercarial sheddings of *F. hepatica* from *L. truncatula* : 1a) numerical distribution of snails with shedding during the patent period; 1b) numerical distribution of metacercariae per snail and per day during the patent period; 1c) correlogram of the cercarial shedding data in fig. 1b. Autocorrelation coefficients are plotted at one day intervals.

Number of waves of shedding	Number of snails involved	Percentage of snails
1	8	7.8
2	7	6.9
3	9	8.8
4	21	20.6
5	16	15.7
6	8	7.8
7	6	5.9
8	6	5.9
9	6	5.9
10	6	5.9
11	3	2.9
12	3	2.9
13	2	2
14	1	1
Total	102	100

Table I. Distribution of snails producing cercariae in relation to the number of waves of shedding.

heavily infected cattle gallbladders at the slaughterhouse of Limoges. They were incubated for 20 days at 20° C in total darkness according to the method described by Ollerenshaw (1971).

Two batches were constituted. Twenty-five controls were not exposed to the parasite. The other 267 snails were individually exposed in 35-mm petri dishes to two miracidia of *F. hepatica* for four hours at 20° C. They were then raised in aquaria for 30 days, with five snails per liter of water. The recipients were placed in an air-conditioned room under the following conditions : a constant temperature of 20° C, and an artificial light source of 3,000 lux intensity at the surface of the aquaria in a 12 h/12 h diurnal rhythm. The snails were fed lettuce *ad libitum* (Hourdin *et al.*, 1993). On day 30, the 25 controls and the 140 survivors from the experimental group were individually monitored in 35-mm petri dishes with 2-3 ml of water per recipient and a piece of lettuce (0.5 cm²). The petri dishes were kept in the same conditions as the aquaria. They were checked daily in the morning, between 10 and 12 a.m. for maximal shedding of cercariae occurred during the night. The water present in the dishes is then changed and a piece of lettuce is added if necessary until the first emission of cercariae. At the onset of shedding, the daily surveillance was pursued, however, a count was performed of the fixed metacercariae, floating cysts. This protocol was followed until snail death.

The individual numbers of metacercariae recorded daily from the infected snails were averaged and their standard deviations determined. The mean daily values were then analyzed by a method of detecting activity rhythms (Broom, 1979; Théron, 1981), such as the autocorrelation test. We have also studied the number of shedding waves during the patent period. Each wave is characterized by the regular emission of 10 cercariae at least during one or several days, and is separated from the subsequent wave by an inter-wave of one day or more.

One-hundred-two snails shed parasites, yielding a frequency of 74 %. Their post-mortem height was 5.7 ± 0.6 mm (as compared with 7.2 ± 1.2 mm in controls). The patent period began on day 44 (± 7.8 days) and lasted 46 ± 27.6 days. The total number of metacercariae shed by all *L. truncatula* was 24,325 of which 23,058 were fixed and 1,267 were floating, yielding a percentage of 95 % and 5 % respectively.

There were 102 snails which shed parasites (fig. 1a) on the first day, and 56 on the second day. Their number subsequently decreased until day 76 to four snails; afterwards, shedding involved one to four snails until day 114. It was thereafter irregular and ceased on day 124.

Changes in the mean numbers of metacercariae per snail and per day are shown in figure 1b. The values were greater during the first 30 days of the patent period and subsequently decreased until day 114 despite the presence of several isolated increases. Subsequently the numbers were low or zero until day 124.

Correlogram of fig. 1c does not show any low-frequency periodicity in the production of *F. hepatica* cercariae. There was no infradian-type rhythm when the *L. truncatula* are raised under controlled conditions.

Table I lists the 102 snails in relation with the number of shedding periods. Fourteen waves were recognized in these snails. Four or five shedding periods were most frequently encountered (20.6 % and 15.7 % of the snails respectively). Each of the other percentages did not exceed 9 %.

The presence of an infradian-type rhythm in cercarial shedding has already been described in several trematode-bearing snails. Periods of high and low production in the daily distribution of cercariae have been reported with a periodicity of 35 days and more in three *Schistosoma* species from *Biomphalaria glabrata* (Théron, 1981), *Bulinus globosus* (Fryer and Probert, 1988), *B. truncatus* (Kechemir and Théron, 1989), and *Planorbium metidjensis* (Mouahid and Théron, 1986, 1987). Another infradian-type rhythm was also found by Audousset *et al.* (1989) in the daily distribution of *F. hepatica* cercariae produced by three colonies of *L. truncatula* raised in seminatural conditions (daily temperature varying between 8° and 22° C) but its periodicity was only seven days.

Our data demonstrate the absence of an infradian-type rhythm in the emission of *F. hepatica* cercariae when *L. truncatula* is raised under constant conditions. However, the experimental design (short acclimatization of snails and daily manipulations) may interfere with biological rhythm so that our results should be taken as preliminary. Another report (Dreyfuss and Rondelaud, 1994) gives also the same result in *L. tomentosa* infected by the same trematode and raised at 23° C. Under these conditions, there is a discordance between these data and those reported by Audousset *et al.* (1989). One explanation would be to attribute the rhythm found by Audousset *et al.* to the experimental conditions used by these authors during their observations on shedding of cercariae. If this assumption is true, then one or several external factors may influence this periodicity (temperature, light,...), as was previously demonstrated during observations by Kendall and McCullough (1951), and by Pêcheur (1966) concerning other characteristics of emission of *F. hepatica* cercariae. Further observations were thus necessary to determine the nature of

the environmental factor(s) which might affect this periodicity.

When infected *L. truncatula* are raised under constant conditions, there is an increase in the number of shedding waves (a total of 14) as in the number of snails which produce their cercariae over several periods. These results do not concord with the number of waves (five or seven) reported by Audousset *et al.* (1989) in *L. truncatula* or by Dreyfuss and Rondelaud (1994) in *L. tomentosa* (at a constant temperature of 23° C). There is likewise a discrepancy in the frequency in which snails shed their parasites in a single wave : 7.8 % in our study, 38.1 % and 50 % respectively for the previously cited authors. These changes in wave pattern can only be ascribed to the constant conditions and the lower breeding temperature used during our experiment.

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