FASCIOLA GIGANTICA : THE PARASITE BURDEN IN *LYMNAEA NATALENSIS* THAT DIED AFTER A CERCARIAL SHEDDING

DREYFUSS G.*, DA COSTA C.**, RAKOTONDRAVAO*** & RONDELAUD D.**

Summary :

Histological studies were carried out in *Lymnaea natalensis* to determine why the number of *Fasciola gigantica* cercariae decreased when the patent period duration increased. Observations were performed in snails killed between days 30 and 135 after exposure, and in snails that died following a cercarial shedding between days 53 and 172 after exposure (at 23 °C). Free and degenerated rediae became increasingly numerous as experiment duration increased; they represented 60 % and more of the redial burden after day 135. Free and degenerated cercariae increased in numbers after day 75, and predominated in older snails.

KEY WORDS : cercaria. redia. *Fasciola gigantica*. *Lymnaea natalensis*.
MOTS CLES : *cercaire. rédie.* Fasciola gigantica. Lymnaea natalensis.

Résumé : FASCIOLA GIGANTICA : LA CHARGE PARASITAIRE CHEZ DES LYMNAEA NATALENSIS MORTES APRES UNE ÉMISSION CERCARIENNE.

Des études histologiques ont été entreprises sur des Lymnaea natalensis *pour déterminer pourquoi le nombre de cercaires de* Fasciola gigantica *diminue lorsque la durée de la période patente augmente. Les observations ont été réalisées chez des limnées sacrifiées entre le 30ᵉ et le 135ᵉ jours d'expérience, ainsi que chez des mollusques morts après une émission cercarienne entre le 53ᵉ et le 172ᵉ jours (23 ° C). Les rédies indépendantes et dégénérées sont de plus en plus nombreuses lorsque la durée de l'expérience augmente; elles représentent plus de 60 % de la charge rédienne après le 135ᵉ jour. Les cercaires indépendantes et dégénérées s'accroissent en nombre après le 75ᵉ jour et prédominent chez les mollusques plus âgés.*

Several studies have already been carried out on the cercarial shedding of *Fasciola gigantica* from the snail *Lymnaea natalensis* (Albaret *et al.*, 1980 ; Cheruiyot and Wamae, 1990). Cercariae are shed during the patent period, however, the number of these larvae per snail and per day was progressively reduced when the life span of infected snails increased (Da Costa *et al.*, 1994). From these data, the question arises to know what happens in these infected *L. natalensis.* To answer this question, we performed an experimental infection of *L. natalensis* by *F. gigantica*, and a comparative histological study between snails killed at regular intervals and those that died following the last cercarial shedding.

The colony of *L. natalensis* originated from Ambanidia and Vohisarika, in the province of Tananarive (Madagascar). The adults were transported to France and placed in covered aquaria (at 23 °C) where they laid eggs. These eggs provided the 4-mm snails that were used in the experiment. The *F. gigantica* eggs were collected regularly at the slaughter-house of Tananarive (Madagascar) from the gall-bladders of heavily infected cattle. They were placed in total darkness for 20 days at 20 °C.

Two groups of snails were constituted. The 150 snails of the first group (living snails) were each exposed for four hours to two *F. gigantica* miracidia and were raised at 23 °C in covered, closed-circuit aquaria, five snails per liter of water. At day 30, they were isolated in 35-mm petri dishes at 23 °C and were observed daily to count metacercariae present in petri dishes. Samples of five *L. truncatula* each were made at the following dates : 45, 60, 75, 90, 105, and 120 days after exposure. Sample of day 135 included only the three last survivors. All the 33 sampled snails had shed cercariae before their sacrifice. They were immersed into Bouin's fixative followed by immediate breaking of the shell. The 483 snails of the second group (dead snails) were exposed to miracidia and raised under previously-described protocol. From these snails, 29 of them were found dying just after a cercarial shedding. Snail death was assured by the absence of reaction following repeated pinching of a tentacle. The 29 snails were also immersed into Bouin's fixative. Serial sections (5 μm thick) were stained using Harris' hematoxylin and modified Gabe's trichrome.

Free rediae and cercariae present in the snail's body were counted depending on their physiological state (whether normal or degenerated). Normal rediae had morulae with round nuclei whereas those of rediae degenerated before snail death had morulae with flat-

* Laboratoire de Parasitologie, Faculté de Pharmacie,
** Laboratoire d'Histopathologie Parasitaire, Faculté de Médecine, 2, rue du Docteur Raymond Marcland, F-87025 Limoges Cedex, France.
*** Département de Recherches Zootechniques et Vétérinaires, Division de Parasitologie, B.P. 4, Tananarive, Madagascar.
Send correspondence to : Dr. D. Rondelaud, Faculté de Médecine, F-87025 Limoges Cedex.- Fax : 55.43.58.93.

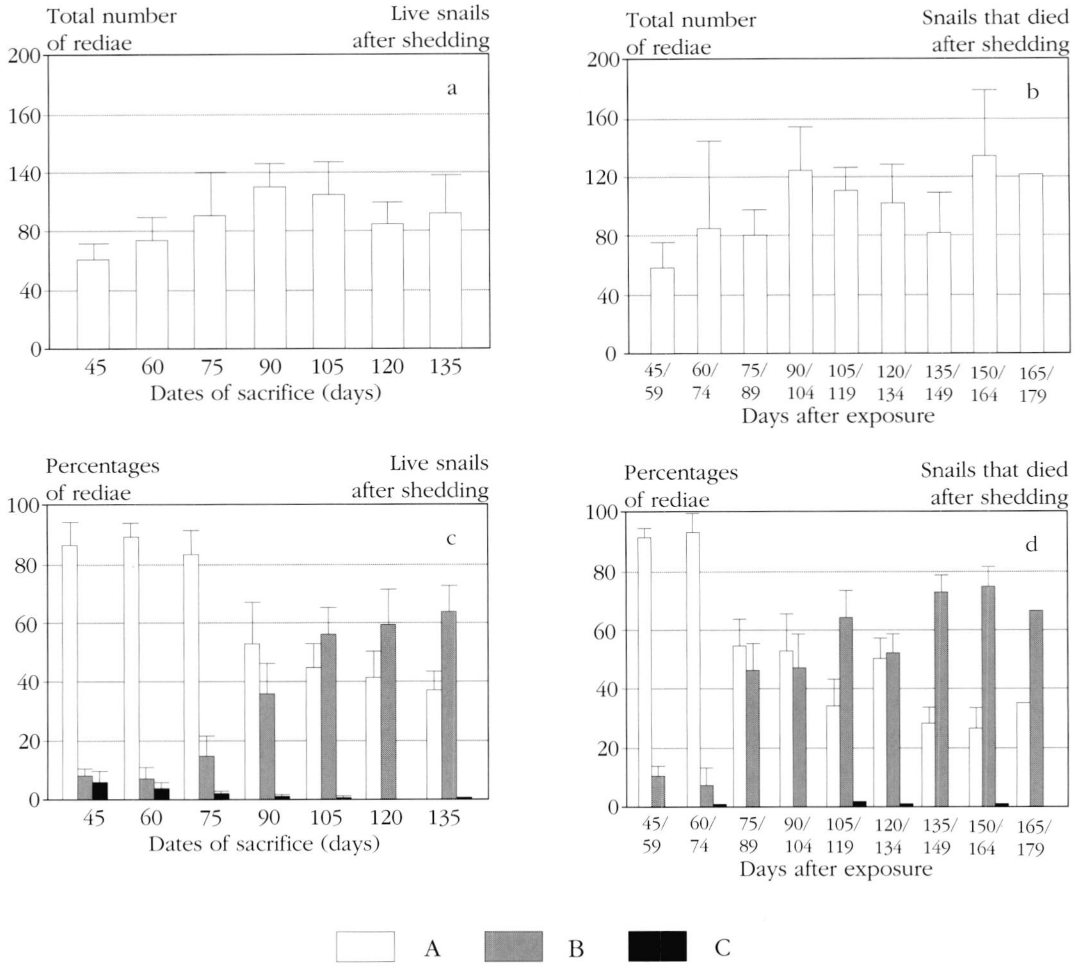

Fig. 1. – *F. gigantica* rediae in *L. natalensis* : i) total number of rediae (1a, b); ii) average number of rediae expressed as percentages in relation with their physiological state (1c, d). The bars presented in graphs correspond to standard deviations. A : normal free rediae; B : degenerated free rediae; C : intraredial rediae.

Dates of sacrifice (days)	45	60	75	90	105	120	135		
Number of sacrificed snails	5	5	5	5	5	5	3		
Number of metacercariae per snail (range)	99-187	135-267	128-232	154-249	162-261	122-274	137-229		
Time intervals (days)	45/59	60/74	75/89	90/104	105/119	120/134	135/149	150/164	165/179
Number of dead snails	3	4	3	4	4	3	4	3	1
Number of metacercariae per snail (range)	121-179	161-248	231-267	137-258	183-244	157-213	249-278	252-281	179 –

Table I. – Numbers of metacercariae in infected live *L. truncatula* and in snails that died after cercarial shedding.

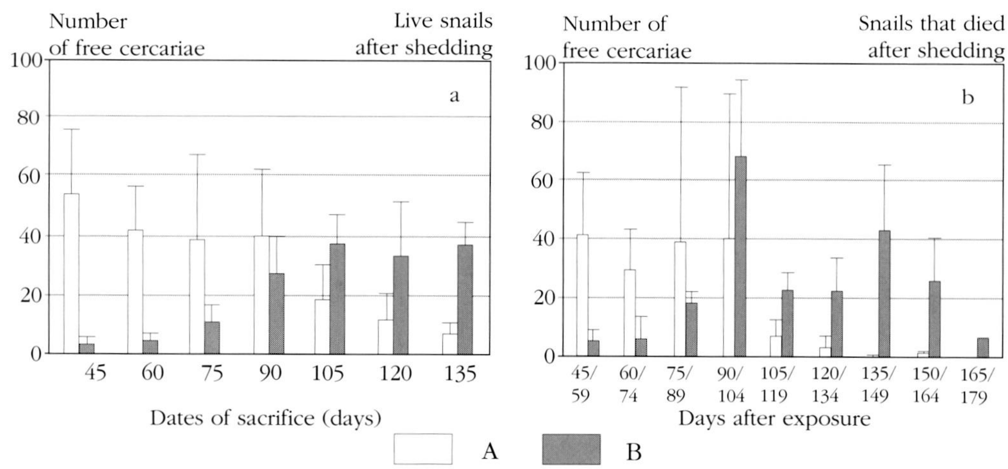

Fig. 2. – Total number of free cercariae per snail in *L. natalensis*. The bars presented in the graphs correspond to standard deviations. A : normal free cercariae; B : degenerated free cercariae.

tened, often triangular and pycnotic nuclei. Cystogenic cells with flattened and pycnotic nuclei characterized free cercariae degenerated before snail death (Rondelaud and Barthe, 1980). These characteristics can be easily distinguished from nucleocytoplasmic clarification and intercellular vacuoles which appeared progressively in parasites after snail death.

The values obtained for these parameters were presented in relation to the date of sacrifice (living snails) and length of snail survival (53 to 172 days), divided into time intervals of 15 days (dead snails). The numbers of degenerated rediae were expressed as a percentage in relation to the total number of these larval forms in the snail. Mean values were determined from the individual values of each parameter and standard deviations were calculated for each of the dates of sacrifice and previously described time intervals. These values were then analyzed by Anova.

Table I indicates distribution of snails and metacercariae over time. The number of cysts per snail ranged from 99 to 274 in living snails, and from 121 to 281 in dead snails. Snail height in the two snail groups ranged from 6 to 12 mm (9 mm on average) at the time of death (data not shown).

Figure 1a, b shows the total number of rediae in relation with experiment duration. In living snails (Fig. 1a), the mean number ranged from 61.2 to 109 rediae whereas it ranged from 57 to 135 rediae in snails died after shedding (Fig. 1b). Significant variation between all mean values was noted in living snails ($p < 0.01$) and dead snails ($p < 0.05$); however, the differences between the mean values of day 60 (or the 60/74 day class) and older samples were not significant.

In both snail groups, the percentage of normal free rediae decreased as experiment duration increased (Fig. 1c, d). For example, in dead snails of the 45/74 day classes it was 90 to 92% whereas in snails of the 135/149 day class and beyond it was 26 to 34 % (Fig. 1d). Inversely, the percentages of degenerated free rediae increased. Using the previous example, there were respectively 6 to 10 % initially, and 65 to 73 % in the older classes. The number of intraredial rediae was 5.4 per snail in living snails at day 45 and decreased subsequently until day 105 (Fig. 1c). In dead snails (fig. 1d), these rediae were rare or absent.

Figure 2 indicates the numbers of free cercariae found in snails of the two groups. The number of normal cercariae per snail ranged from 39 to 54 in living snails from day 45 to day 90 (Fig. 2a), and from 29 to 41 in dead snails 45 to 104 days of age (Fig. 2b). In older snails, they were fewer or none. Significant variation between the mean values at the different dates of sacrifice was found in living snails ($F = 5.07$: $p < 0.01$). This variation between the mean values in the different time intervals was also significant in dead snails ($F = 4.1$; $p < 0.01$). Degenerated cercariae increased in number after day 75 in snails of both groups (Fig. 2a, b). In the older snails, this form was the most frequently encountered, and the mean values ranged from 27 to 37 cercariae in living snails, from 25 to 65 in dead snails. Significant variation between the mean values at the different dates of sacrifice was noted in living snails ($F = 3.94$; $p < 0.01$) but the differences between the mean values were not significant in dead snails.

Small numbers of internal metacercariae were present in living snails as in dead snails after day 60 (0.7 to 2.5 per snail). Considerably more were present in the 150/164 day class (9.9 per snail in the second group; data not shown).

Degenerated rediae have already been reported in *L. natalensis* by Dinnik and Dinnik (1956), involving 6 % of the rediae beginning at day 120 in snails exposed to a single miracidium. In snails exposed to three, four, and five miracidia, 10 % were involved at day 119, and 17 % at day 168. Contrary to these authors, the percentages in this study were higher (in the 75/89 day class, 45 % were degenerated rediae), and subsequently rose even more (affecting 65 to 73 % of the rediae beginning at day 135). In *L. truncatula* infected by the same trematode, Rakotondravao *et al.* (1992) reported similar findings : 12 to 16 % were degenerated rediae between days 28 and 60. The discrepancy between the percentages of Dinnik and Dinnik and the other data can partially be explained by the technique employed in this study since signs of degeneration can be detected earlier histologically than by simple dissection of snails. The most likely hypothesis for explaining the high percentage of degenerated rediae at the end of our experiment concerns intermediate host internal volume available for parasite development : a snail can only sustain a certain number of parasites, and degeneration would occur as a self-regulating process to eliminate an excessive number of parasites.

Our results also demonstrate that degenerated free cercariae were found at a later date than that of rediae, since a substantial reduction in the number of normal free cercariae was observed beginning at day 105 of the experiment whereas a corresponding rise in degenerated parasites occurred after day 75. If the previously cited hypothesis is accepted, it is logical to suggest that free cercariae are more resistant than rediae when placed in unfavorable conditions. This may also provide an explanation for internal cyst formation which would represent a process that permits certain cercariae to survive under adverse host conditions. The relatively small number of these internal cysts may be interpreted in the light of observations by Vareille-Morel *et al.* (1993) when it was noted that formation of these metacercariae only occurred during the last moments of the snail's life.

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