

Fasciola gigantica*: larval productivity of three different miracidial isolates in the snail *Lymnaea truncatula

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Abstract

Bimiracidial infections of *Lymnaea truncatula* with three isolates of *Fasciola gigantica*, originating from China, Egypt and Madagascar, were carried out to determine the effect of geographic origin of the parasite on the larval productivity of redial generations. The prevalences of experimental infections in snails exposed to strains from Madagascar, China and Egypt were 20.8%, 60.0% and 80.0%, respectively. At day 49 post-exposure (p.e.), the total number of free rediae in snails infected with the Egyptian isolate was significantly higher than that recorded in the Madagascan group. On the other hand, at day 49 p.e., the majority of cercariae in the Chinese and Egyptian groups were produced by R2a rediae (70.6% and 66.6% of cercariae produced by all live rediae), while, in the Madagascan group, the cercariae were produced mainly by the first redial generation. Snails infected with the Egyptian isolate of miracidia developed more live rediae and, consequently, could produce a higher number of cercariae. As a result, *L. truncatula* snails were highly adapted to infections with the Egyptian and Chinese isolates of *F. gigantica*.

Introduction

The snail *Lymnaea truncatula* is known to support larval development of *Fasciola gigantica* in the field (Dinnik & Dinnik, 1956/1957) and in the laboratory (Boray, 1978; Dreyfuss & Rondelaud, 1997). In this intermediate host, up to three redial generations succeed one another (Rakotondravao *et al.*, 1992; Dar *et al.*, 2002). However, these authors found a slow larval development of *F. gigantica* in French populations of *L. truncatula* when they used Madagascan isolates of miracidia for their experiments. This finding poses the question of whether the geographic origin of miracidial isolates has an influence on subsequent larval development of redial generations and their cercarial productivity.

According to Boray (1969), great variation in the susceptibility of *L. truncatula* populations to *F. gigantica*

infections was noted in relation to the origin of flukes. Using German snails, Boray found a disparity between *L. truncatula* and *F. gigantica* in the first passage, and estimated that the adaptation of this trematode to the intermediate host might be obtained by laboratory passages. As no information on the effect of this factor on the development of *F. gigantica* redial generations was available, the following questions arose: (i) does the geographical origin of flukes have a significant influence on the redial burden within the snail body; and (ii) what is the effect of this factor on the cercarial productivity of each redial generation? In an attempt to answer these questions, three groups of the same population of *L. truncatula* were subjected to bimiracidial infections with three different isolates of *F. gigantica*, originating from China, Egypt and Madagascar.

Materials and methods

The population of *L. truncatula* originated from Courcelles, commune of Saint Michel de Veisse, department

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of Creuse, central France. As the definitive host may influence the rate of subsequent development of *F. gigantica* in snails (Al-Kubaisee & Altaif, 1989), the eggs of this trematode were only collected from the gall bladders of naturally infected cattle originating from China (province of Guang-Xi, abattoir of Nanning), Egypt (Al-Gharbia governorate, abattoir of Tanta), or Madagascar (abattoir of Tananarive). Eggs were incubated for 20 days at 20°C in total darkness as previously described by Ollerenshaw (1971) for eggs of *F. hepatica*. Snails, measuring 4 mm in height, were divided into three groups of 100 snails each. Each snail from the first group was subjected to a bimiracidial infection with the Madagascan isolate, while the *L. truncatula* from the second and the third groups were individually exposed to bimiracidial infections with Chinese and Egyptian isolates, respectively. They were then raised in open aerated breeding boxes (50 snails per box) for 49 days at 20°C under an experimental photophase of 12 h (Dar *et al.*, 2002). Samples of 5 to 10 snails each were taken among the survivors on the following days: 14, 21, 28, 35, 42 and 49 post-exposure (p.e.). Snails were dissected in tap water under a stereomicroscope to detect live rediae. The total number of rediae and that of intraredial developmental stages (morulae, embryos, daughter rediae, procercariae, and cercariae) were determined.

The criteria used to identify redial generations of *F. gigantica* are based on the combination of the following factors: age of infection, the development of intraredial developmental stages, and the general form of the pharynx (Rakotondravao *et al.*, 1992). These rediae were classified into the following categories: first-appearing mother redia from the sporocyst (R1a), second-appearing mother rediae from the sporocyst (R1b), daughter rediae from R1a (R2a), and daughter rediae from R1b and R2a (R2b/R3a).

The four first parameters studied were the mortality rate of snails at day 30 post-exposure (p.e.), the infection rate of snails with *F. gigantica*, the total number of free rediae per snail, and the number of rediae for each redial category. A comparison test of experimental frequencies and a t-test (Stat-Itcf, 1988) were used to establish levels of significance. The cercarial productivity in each redial category (Augot & Rondelaud, 2001) was the fourth parameter. This was estimated using the difference between the highest mean number of morulae (N1) counted in free rediae (the date varied with the redial category) and another number (N2) which was obtained by adding the intraredial developmental stages (morulae, procercarial embryos, and procercariae) at day 49 p.e., and the highest mean number of proredial embryos and daughter rediae in the course of infection (the date varied with the redial category).

Results

The mortality rate of Egyptian snails at day 30 p.e. was significantly lower ($P < 0.05$) than those noted in Chinese and Madagascan groups (10.0% instead of 39.8% and 47.2%, respectively). The prevalence of infection was significantly higher ($P < 0.05$) in the Egyptian and Chinese groups (80.0% and 60.0% instead of 20.8% in Madagascan snails).

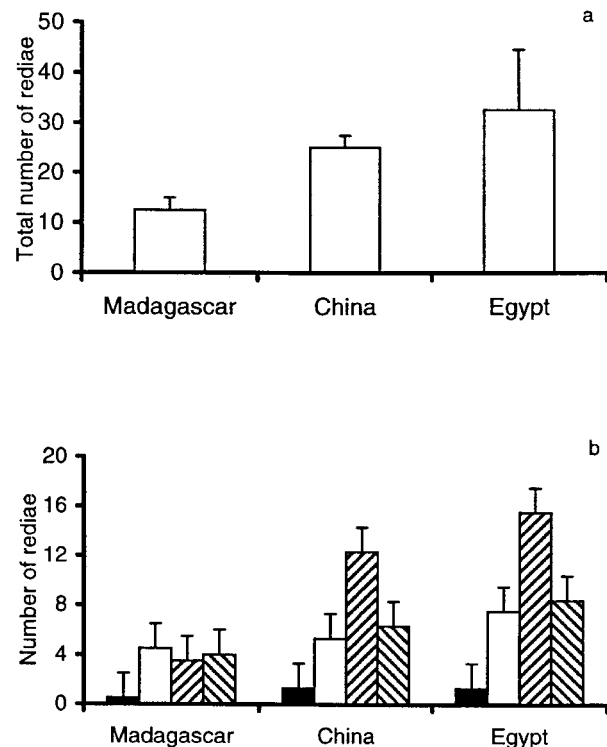


Fig. 1. Mean values and SD for redial generations of three isolates of *Fasciola gigantica* from China, Egypt and Madagascar, in *Lymnaea truncatula* at day 49 post-exposure: (a) the total number of rediae and (b) the number of rediae for each category (■, R1a; □, R1b, ▨, R2a, or ▩, R2b/R3a). See table 1 for explanation of redial categories.

The total number of rediae at day 49 p.e. (fig. 1a) was significantly higher ($t = 3.30$, $P < 0.05$) in snails infected with Egyptian miracidia than in the Madagascan group. Another significant difference ($t = 7.36$, $P < 0.001$) was also found between the Chinese and Madagascan groups. The mean number of R1a rediae (fig. 1b) found in the three snail groups at day 49 p.e. did not significantly differ. A similar finding was also noted for the number of R1b rediae. Compared to Madagascan snails, the mean number of R2a rediae in the Chinese ($t = 5.71$, $P < 0.01$) and Egyptian ($t = 3.76$, $P < 0.05$) groups was significantly higher. The number of R2b/R3a rediae was slightly higher in the Chinese and Egyptian snails than in the other group. However, no significant differences between these mean numbers were found.

Table 1 shows, for each live redia at day 49 p.e., the number of morulae which differentiated into cercariae in the three groups of snails. In snails infected with the Chinese and Egyptian isolates, the majority of cercariae were produced by R2a rediae (6.4 and 11.4 cercariae per redia, respectively) and corresponded to 70.6% and 66.6% of cercariae produced by all live rediae. In the Madagascan group, cercariae were produced by the R1a (41.5% of cercariae) and R1b (58.5%) rediae. If the numbers of cercariae produced by each redial category are added, each snail from the Egyptian group harboured a total of 258.6 cercariae at day 49 p.e., whereas the mean

Table 1. *Fasciola gigantica*: cercarial productivity of redial generations in *Lymnaea truncatula* for each miracidial isolate.

Origin of parasite	Redial category	Mean number of free rediae at day 49 p.e.	Numbers of morulae differentiated into cercariae		Numbers of cercariae produced by live rediae in a snail (and %)
			N1–N2*	Number per redia	
Madagascar	R1a	0.5	13.0–6.0	7.0	3.5 (41.5)
	R1b	4.5	8.4–7.3	1.1	4.9 (58.5)
China	R1a	1.3	15.0–7.0	8.0	10.4 (9.4)
	R1b	5.3	11.5–7.3	4.2	22.3 (20.0)
	R2a	12.3	12.3–5.9	6.4	78.8 (70.6)
Egypt	R1a	1.3	10.8–5.4	5.4	7.0 (2.7)
	R1b	7.5	13.6–3.0	10.6	79.5 (30.7)
	R2a	15.5	16.9–5.8	11.1	172.1 (66.6)

*N1, the highest number of morulae in each redia during the experiment; N2, mean number of developmental stages remaining in the rediae at day 49 p.e. (the daughter rediae are also counted in N2); R1a, first-appearing mother redia from the sporocyst; R1b, second-appearing mother rediae from the sporocyst; R2a, daughter rediae from R1a.

production of cercariae was only 111.4 and 8.5 cercariae per snail in the Chinese and Madagascan groups, respectively.

Discussion

Compared to the Madagascan origin of miracidia, snails infected with the Chinese and Egyptian isolates developed more live rediae and, consequently, could produce higher numbers of cercariae. Although the developmental patterns of redial generations were nearly identical in these three snail groups, the R2a rediae developed rapidly in the Chinese and Egyptian groups, and produced the majority of cercariae. These results indicated that these two miracidial isolates are highly adapted to the French population of *L. truncatula* used in this experiment. As *L. truncatula* snails were naturally present in Egypt (Brown, 1994), they may play a role in transmitting the parasite, in addition to *L. natalensis* which is the principal intermediate host of *F. gigantica* in Egypt. On the other hand, only *L. natalensis* is found in Madagascar (Stothard *et al.*, 2000) and this leads to the assumption that *L. truncatula* is an unsuitable intermediate host for the Madagascan isolate of *F. gigantica*. As the development and productivity of redial generations are influenced by the species of the intermediate host (Rakotondravao *et al.*, 1992), rediae are also affected by the geographical origin of *F. gigantica*.

The greater role of R2a rediae in cercarial productivity of *F. gigantica* in Chinese and Egyptian snails was in agreement with that reported by Augot & Rondelaud (2001) for the cercarial productivity of *F. hepatica* rediae during the normal development of redial generations. As the number of live R2a rediae depended on the behaviour of R1a rediae, as demonstrated by Augot *et al.* (1998, 1999) for *F. hepatica*, the results found in the present experiment indicated that all R1a rediae of *F. gigantica* from the Chinese and Egyptian snails remained alive during the course of infection within the body of the snail and, consequently, induced an usual development of subsequent redial generations (Rakotondravao *et al.*, 1992). To

explain this last finding, the more likely hypothesis is to admit the absence of competition between developing larvae within the snail body during the first weeks of the experiment, so that the two miracidia used to infect each snail would transform into sporocysts and would each produce a R1a redia which remained alive in the course of infection and might produce a higher number of R2a rediae.

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