Journal of Helminthology (2004) **78,** 215–218 DOI: 10.1079/JOH2003224

Larval productivity of *Fasciola gigantica* in two lymnaeid snails

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Abstract

Two groups of *Galba truncatula* and two groups of *Lymnaea natalensis* were experimentally infected with *Fasciola gigantica* to determine if snail species had an influence on the redial burden and cercarial shedding of this trematode when snails of both species were infected with the same isolate of miracidia. In the two groups used for the study of redial burden, the total number of free rediae was significantly higher at day 49 post-exposure in *L. natalensis* than in *G. truncatula*. In the groups used for cercarial shedding, the life-span of cercaria-shedding snails and those of infected snails which died without cercarial emission, and the duration of the prepatent period were significantly longer in *L. natalensis* than those noted in *G. truncatula*. However, the mean numbers of shed cercariae did not significantly differ and showed no differences in their daily distribution throughout the shedding period. These results demonstrate that *G. truncatula* might be the principal intermediate host of *F. gigantica* in Egypt, at least in the areas where this lymnaeid species lives.

Introduction

Lymnaea natalensis is known to be the habitual intermediate host of *Fasciola gigantica* in Egypt, especially in waterbodies of the Nile Delta (Farag, 1998). Another lymnaeid species, *Galba truncatula*, can sustain full larval development of *F. gigantica* in the field (El-Shazly *et al.*, 2002) and in the laboratory (Dar *et al.*, 2003). Even though another lymnaeid snail, *L. columella* (Ahmed & Ramzy, 1999) and a planorbid species, *Biomphalaria alexandrina* (Farag & El-Sayad, 1995), are also naturally infected with *F. gigantica* in Egypt, *L. natalensis* and *G. truncatula* are responsible for transmitting fasciolosis in most areas of this country (Farag, 1998; El-Shazly *et al.*, 2002). The developmental pattern of *F. gigantica* redial generations in both these lymnaeids is identical, but the larval production of this trematode in *L. natalensis* (Dinnik & Dinnik, 1956) differs from that found in *G. truncatula* (Rakotondravao *et al.*, 1992; Dar *et al.*, 2002). This difference might be due to the isolates of miracidia used, as Dar *et al.* (2003), using three different isolates of *F. gigantica* originating from China, Egypt and Madagascar, found that larval production was influenced by the origin of the parasite. In addition, differences in larval production might be due to the species of snails used, their susceptibility to *Fasciola* infections, and their growth during the experiments, as demonstrated by Vignoles *et al.* (2002) for *F. hepatica*. As no comparative studies have hitherto been undertaken on *G. truncatula* and *L. natalensis* infected with *F. gigantica*, it would be logical to determine if the species of snails had an influence on the larval productivity of this trematode when snails of both species were infected with the same isolate of miracidia under constant experimental conditions. Therefore, in an attempt to answer this question, experimental infections of *G. truncatula* or *L. natalensis* by *F. gigantica* were performed to (i) count free rediae inside dissected snails and (ii) determine the number of cercariae shed by other infected snails until their death.

Materials and methods

The population of *G. truncatula* originated from Berneuil, department of Haute Vienne (central France).

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Snails from this population are known to be devoid of any natural infections because of repeated collections of adult snails over the previous six months and the absence of larval digeneans in dissected snails. A total of 100 snails, measuring 4 mm in height, were collected from this site and transported to the laboratory before being placed in breeding aquaria at 20°C. The population of *L. natalensis* originated from the El-Mansoria river, Giza governorate, Egypt. Adult snails were reared under laboratory conditions to provide the 4-mm high snails used in this experiment. Eggs of *F. gigantica*, collected from the fluid inside the gall-bladders of naturally-infected cattle at the slaughterhouse of Tanta, Gharbia governorate, Egypt, were incubated at 20°C for 20 days in the dark according to the method used by Ollerenshaw (1971) for the eggs of *F. hepatica*.

Four groups of 50 snails each (two for *G. truncatula*, and two for *L. natalensis*) were used. All snails were individually exposed to bimiracidial infections with *F. gigantica* for 4 h, then raised in open aerated boxes (50 snails per box) and fed cos lettuce *ad libitum*. These boxes were placed in air-conditioned rooms at 20°C (for *G. truncatula*) or at 22–25°C (for *L. natalensis*), with a diurnal photophase of 3000–4000 lux light intensity. Samples of 5–10 snails each were randomly taken among the survivors in the first group of *G. truncatula* or in the second of *L. natalensis* for each of the following dates: 14, 21, 28, 35, 42 and 49 days post-exposure (p.e.). No sampling was carried out after day 49 p.e. due to the difficulties in identifying the third and subsequent generations of *F. gigantica* rediae in *L. natalensis*. Snails were dissected in tap water under a stereomicroscope to detect live rediae. Four categories of *F. gigantica* rediae were identified according to the age of infection, the development of intraredial germinal embryos, and the general form of their pharynx (Dar *et al.*, 2003): first-appearing mother redia from the sporocyst (R1a), other mother rediae from the sporocyst (R1b), daughter rediae from R1a (R2a), daughter rediae from R1b (R2b), and granddaughter rediae from R1a (R3a). As R2b and R3a rediae could not be differentiated by the morphology of the pharynx, they were pooled into a single group: R2b/R3a.

On day 30 p.e., the surviving *G. truncatula* from the third group and *L. natalensis* from the fourth group were individually placed in 35-mm diameter Petri dishes, with 2–3 ml of spring water and a piece of cos lettuce per dish. As the shell height of the latter snails increased during the experiment, each snail was placed in a 50-mm diameter Petri dish. Metacercariae were counted on a daily basis until the death of the snail, and the water was changed daily.

Two parameters were determined in the two snail groups used for redial counts: the total number of free rediae per snail and the number of free rediae in each redial category. Seven parameters were measured in the two groups used for the study of cercarial shedding. Three parameters were the survival of snails at day 30 p.e., the frequency of each snail subgroup (cercariae-shedding snails (CS snails); infected snails died without cercarial shedding (NCS snails); and uninfected snails (UNI snails)), and the respective life span of each snail subgroup (between miracidial exposure and snail death). The other four parameters were calculated only for CS snails: the length of the prepatent period (between miracidial exposure and the first day of shedding), that of the whole shedding period, the number of cercariae which exited from each CS snail, and the number of metacercariae for each day of the shedding period. A Pearson correlation test, a Chi-square test, and a Student t-test (Stat-Itcf, 1998) were used to determine levels of significance.

Results

In the two groups of snails used for the study of redial burdens, the prevalences of experimental infections were 60% in both *G. truncatula* and *L. natalensis*, with 616 and 573 rediae being recovered, respectively. The total number of rediae (fig. 1a) in *G. truncatula* significantly ($r = 0.92$, $P < 0.01$) increased as the experiment progressed, as did those in *L. natalensis* ($r = 0.96$, $P < 0.01$). The number of rediae in both snail species was similar up to day 35 p.e. After this date, the quick increase in height of *L. natalensis* resulted in a rapid raise in the number of rediae in this snail, so that the number of rediae was significantly higher ($t = 78.85$, $P < 0.001$) on day 49 p.e. in *L. natalensis* than in *G. truncatula* (a mean of 92.7 rediae at day 49 instead of 42.3). The mean values of R1a rediae (fig. 1b) did not significantly differ in the two snail species. Compared to *G. truncatula*, the mean values of R1b ($t = 16.70$, $P < 0.001$), R2a ($t = 27.95$, $P < 0.001$), and

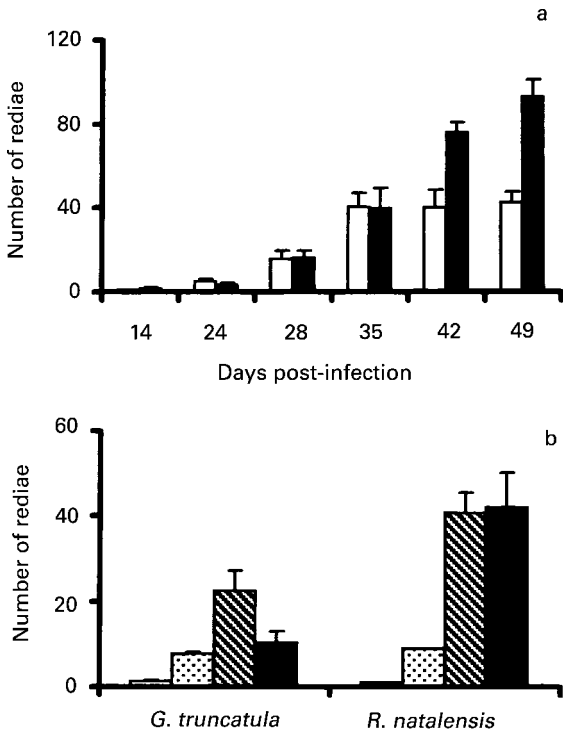

Fig. 1. Mean values and S.D. for (a) the total number of free rediae throughout experiment in both *Galba truncatula* (□) and *Lymnaea natalensis* (■), and (b) the number of free rediae for each category (□, R1a; ▨, R1b; ▧, R2a; ■, R2b/R3a).

https://doi.org/10.1079/JOH2003224 Published online by Cambridge University Press

Table 1. Characteristics of *Fasciola gigantica* infections in the lymnaeid snails *Galba truncatula* and *Lymnaea natalensis* used for the study on cercarial shedding.

Snail species	*Galba truncatula*	*Lymnaea natalensis*
No. of snails at the onset of experiment	50	50
No. of surviving snails at day 30 p.e. (%)	40 (80.0)	49 (98.0)
No. of snails (%)		
CS snails	17 (42.5)	29 (59.2)
NCS snails	5 (12.5)	3 (6.1)
UNI snails	18 (45.0)	17 (34.7)
Life spans (days)		
CS snails	80.0 ± 21.0	100.5 ± 13.5
NCS snails	38.0 ± 10.8	54.0 ± 1.6
UNI snails	74.0 ± 34.2	136.6 ± 1.6
Prepatent period (days)	47.1 ± 4.0	62.7 ± 4.4
Shedding period (days)	32.9 ± 21.8	37.8 ± 14.0
No. of cercariae per CS snail	240.0 ± 152.0	286.3 ± 87.9

CS, cercariae-shedding snails; NCS, infected snails without cercarial shedding; UNI, uninfected snails.

R2b/R3a ($t = 37.80$, $P < 0.001$) rediae were significantly higher in *L. natalensis*.

Table 1 gives the results in the two groups of snails used for the study of cercarial shedding. On day 30 p.e., there was a significant difference ($P < 0.05$) between the survival rates of both snail species. Compared to the values recorded in *G. truncatula*, the life span of CS snails ($t = 3.61$, $P < 0.01$), NCS snails ($t = 3.25$, $P < 0.05$) and UNI snails ($t = 7.75$, $P < 0.001$), and the duration of the prepatent period ($t = 12.30$, $P < 0.01$) were significantly longer in the case of *L. natalensis*. In contrast, the length of the shedding period and the number of cercariae showed no significant differences between the two snail groups.

Figure 2 shows the daily distribution of metacercarial numbers throughout the shedding period for each species of CS snails. In *G. truncatula* (fig. 2a), the mean number of metacercariae progressively increased until day 7 (at 29 cysts per snail) and subsequently declined until day 57. In *L. natalensis* (fig. 2b), the highest mean values (20–22 cysts per snail) were observed between days 3 and 6. Other peaks were also noted on days 10, 14 and 19 (a mean of 21.9, 20.2 and 21.4 cysts per snail, respectively). Afterwards, the values gradually decreased until day 65.

Discussion

The present study demonstrated a significantly greater production of free rediae in *L. natalensis* (mainly in the R2a and R2b/R3a categories), whereas the mean number of shed cercariae does not significantly differ between both species of snails. The higher number of rediae developed in *L. natalensis* was similar to that reported by Dinnik & Dinnik (1956). This might be explained by the faster growth of this snail species over time and the higher shell height of adults (a mean of 22 mm instead of 12 mm for *G. truncatula*: Brown, 1994), as Zischke (1967) and Rondelaud & Barthe (1987) found a positive relationship between the number of rediae produced and the size of the snail host. The similarity in cercarial production of *F. gigantica* by both snail species is more difficult to interpret. Indeed, the number of cercariae produced by *G. truncatula* was within the range values for cercarial production of *F. gigantica* given by Dreyfuss & Rondelaud (1997) or by Dar *et al.* (2003). In contrast, the mean number of larvae recorded for *L. natalensis* was either similar to that reported by Cheruiyot & Wamae (1990) and Da Costa *et al.* (1994), or lower than that recorded by Guralp *et al.* in 1964 (up to 7179 cercariae for a single snail) and by

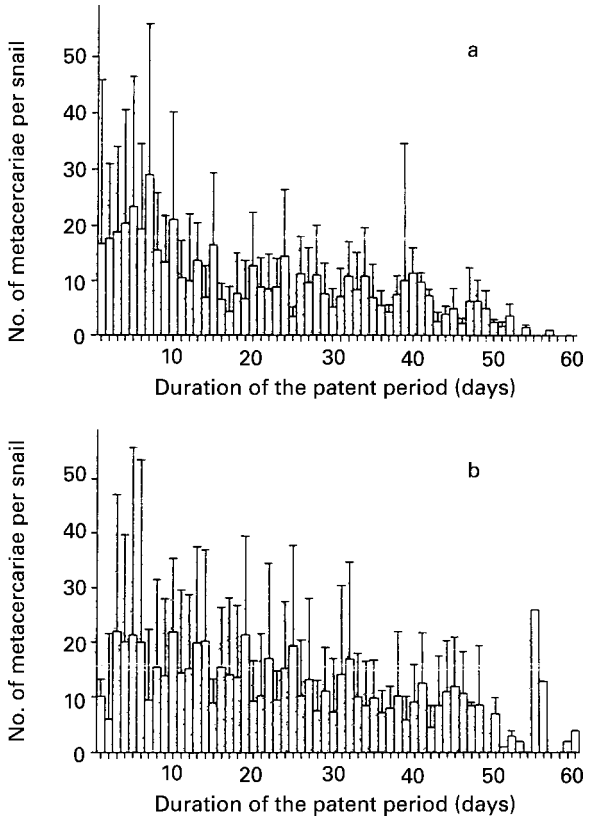

Fig. 2. Mean values and S.D. for the number of *Fasciola gigantica* cercariae shed for each day of the shedding period: (a) *Galba truncatula* and (b) *Lymnaea natalensis*.

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Bitakaramire in 1968 (a mean of 653 per snail). These differences between cercarial productions of *F. gigantica* from *L. natalensis* might partly be due to the geographical origin of the miracidial isolate used for experimental infections (Boray, 1969; Mohamed *et al.*, 1998; Dar *et al.*, 2002), or to the definitive host from which *F. gigantica* eggs and, consequently, miracidia originated (Al-Kubaisee & Altaif, 1989). However, variability in the susceptibility of *L. natalensis* populations to *F. gigantica* infections cannot be excluded.

A comparison of redial burdens of *F. gigantica* in both lymnaeid species with the respective number of cercariae produced demonstrated an excess of free rediae in the case of *L. natalensis*. Indeed, the large numbers of free rediae developing in *L. natalensis* (a mean of 92.7 at day 49 p.e.) resulted only in a cercarial production close to that in *G. truncatula*, in which a mean of 42.3 rediae were recorded on day 49 p.e. Under these conditions, it is likely that *L. natalensis* is the more suitable host for the larval development of *F. gigantica* in Egypt. An argument in support of this is the longer duration of the prepatent period in *L. natalensis* than in *G. truncatula* (table 1). Even though there was a wide range of freshwater species as potential intermediate hosts in the life cycle of *F. gigantica* (Spithill *et al.*, 1999), the present results suggest that *G. truncatula* might be a principal intermediate host of this trematode in Egypt, at least in areas where this lymnaeid species lives. Further studies are needed to verify this hypothesis by infecting local populations of *G. truncatula* and/or *L. natalensis* with local isolates of *F. gigantica* using the protocol of Mohamed *et al.* (1998) in Egypt.

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(Accepted 20 October 2003)