Radix natalensis (Gastropoda: Lymnaeidae), a potential intermediate host of *Fasciola hepatica* in Egypt

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Summary:

Experimental infections of Egyptian *Radix natalensis* with French miracidia of *Fasciola hepatica* were carried out to determine if this snail might act as an intermediate host in the life cycle of this digenean in Egypt. Single exposures of *R. natalensis* to miracidia (2/snail) and two successive exposures (a total of 4 miracidia/snail) were performed using lymnaeids measuring 1 to 6 mm in height. Live larval forms of *F. hepatica* were noted in single- and double-exposed snails. In double exposures, a significant increase of snail survival on day 28 post-exposure (at 24 °C) and an decrease in prevalence were noted when the height of snails at exposure was increasing. Cercariae of *F. hepatica* were shed by these snails (90.7/snail) during a mean patent period of 24.3 days. All snails have released these cercariae during 2-13 waves of shedding. According to these results, *R. natalensis* can be considered a potential intermediate host of *F. hepatica* in Egypt.

KEY WORDS: *Fasciola hepatica*, *Radix natalensis*, cercaria, experimental infections, Egypt.

Résumé : *Radix natalensis* (Gasteropoda : Lymnaeidae), un hôte intermédiaire potentiel de *Fasciola hepatica* en Égypte

Des infestations expérimentales de Radix natalensis *provenant d'Égypte avec un isolat français de* Fasciola hepatica *ont été réalisées pour déterminer si ce mollusque pouvait intervenir comme hôte intermédiaire dans le cycle évolutif de ce Digène en Égypte. Des limnées hautes de 1 à 6 mm ont été soumises individuellement, soit à une seule exposition (2 miracidiums/mollusque), soit à deux expositions successives (4 miracidiums au total par limnée). Dans les deux cas, les mollusques ont présenté des formes larvaires en vie, quel que soit le nombre d'expositions. Chez les limnées soumises deux fois au parasite, on note une augmentation significative de la survie au 28e jour d'exposition (à 24 °C) et une diminution de la prévalence lorsque la hauteur des mollusques lors de l'exposition s'accroît. Des cercaires (90,7 par mollusque) ont été obtenues au cours d'une période patente moyenne de 24,3 jours et ces larves ont été relâchées pendant deux à 13 vagues d'émission. D'après ces données,* R. natalensis *peut être considéré comme un hôte intermédiaire potentiel de* F. hepatica *en Égypte.*

Mots clés : Fasciola hepatica, Radix natalensis, *cercaire, infestations expérimentales, Égypte.*

INTRODUCTION

In Egypt, two liver flukes are reported in the literature (Torgerson & Claxton, 1999). *Fasciola gigantica* is considered to be the indigenous species of *Fasciola* found in the Nile Delta, while *F. hepatica* is present only in imported animals (Lotfy *et al.*, 2002). According to Brown (1994), the intermediate hosts of both *Fasciola* exist in Egypt. *Radix natalensis* (= *R. caillaudi*), *Pseudosuccinea columella* and, at a lesser extent, *Biomphalaria alexandrina* are known to be natural hosts of *F. gigantica* (Farag & El Sayad, 1995; Ahmed & Ramzy, 1999; El-Dafrawy, 2002; Hussein & Khalifa, 2008). In the case of *Galba truncatula*, successful infections with both *Fasciola* species were obtained in the laboratory (Dar *et al.*, 2003a, b, 2004) but only natural infections with *F. gigantica* (Dar *et al.*, 2005) or with *Fasciola sp.* (El-Shazly *et al.*, 2002) were noted in the field. As the distribution of *P. columella* and that of *G. truncatula* seem limited in northern Egypt (Dar, unpublished data), one may wonder if *R. natalensis* would not be a local intermediate host in the life-cycle of *F. hepatica*. A literature review on this last point demonstrated the existence of conflicting results. Negative infections of *R. natalensis* with *F. hepatica* were reported by several authors (Kendall, 1965; Boray, 1966; Mohamed *et al.*, 1998; Hussein & Khalifa, 2008). In contrast, in another experiment, Boray (1969) obtained successful infections of young snails and the production of viable cercariae when he used a Kenyan population of *R. natalensis* and European isolates of *F. hepatica*. In the same way, a 4.2 % infection rate and the shedding of cercariae were reported by Dreyfuss (1994) during experimental infections of Malagasy *R. natalensis* with a French isolate of *F. hepatica*. In view of these findings, the following questions arose: can *R. natalensis* sustain the larval

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development of *F. hepatica*? Are all stages of snail's life susceptible to *F. hepatica* infection? How many cercariae of *F. hepatica* the snail can shed and what is the dynamics of cercarial emission during the patent period? To answer these questions, two experiments were carried out by subjecting an Egyptian population of *R. natalensis* to *F. hepatica* miracidia. As both species of *Fasciola* and intermediate forms exist in Egypt (Periago *et al.*, 2008), the parasite isolate used for this study originated from central France. The results obtained in the present study were also compared to the report by Dar *et al.* (2004) because this Egyptian population of *R. natalensis* had already been used for experimental infections with sympatric isolates of *F. gigantica*.

MATERIALS AND METHODS

Snails and parasite

The population of *R. natalensis* was living in an irrigation canal at El Mansoria, Giza governorate, Nile Delta. As another lymnaeid (*P. columella*) was also living in the same governorate (Ahmed & Ramzy, 1999), the identification of *R. natalensis* was confirmed via the examination of the genital apparatus (Hubendick, 1951) and that of the periostracum (Brown, 1994; Pointier *et al.*, 2007). No species identification using the available molecular tools (Stothard *et al.*, 2000; Bargues *et al.*, 2001) was made in the present study. Snails measuring 1-1.5 cm in height were collected from this canal in July 2002 and were placed in aerated aquaria kept at a constant temperature of 24 °C. This laboratory strain was used for later experimental infections. To verify if the susceptibility of this snail strain to *F. gigantica* had not changed over time since July 2002, a preliminary experiment was carried out in September 2008 by subjecting 4-mm-high snails to individual bimiracidial exposures with this digenean. The values noted for the prevalence of snail infection and the number of shed cercariae (data not shown) were close to those reported by Dar *et al.* (2004), thus demonstrating that the snail susceptibility to *F. gigantica* infection was the same in spite of several years of snail breeding in the laboratory.

The eggs of *F. hepatica* were collected from the gall bladders of heavily infected cattle at the slaughterhouse of Limoges, department of Haute Vienne, central France. They were washed several times with spring water and were incubated at 20°C for 20 days in the dark (Ollerenshaw, 1971).

Experimental protocol

Table I gives the number of experiments (A, or B), the number of bimiracidial exposures for each group, the shell height of snails at exposure, and the quantity of snails in each group. Experiment A was performed to determine snail survival and the prevalence of infection by dissecting surviving lymnaeids at day 28 post-exposure (p.e.). A first group of *R. natalensis*, measuring 1-2 mm in height, was once subjected to individual bimiracidial exposures with *F. hepatica*. However, the prevalence of infection was low in this case (12.0 %, see Table I) so that the snails from the other five groups were each submitted to two successive bimiracidial exposures (with a 4-hour interval between them). This method was chosen according to the report by Boray (1969). Indeed, this author noted an increase in the prevalence of infection when snails (*Lymnaea peregra* in this case) were subjected to successive exposures with *F. hepatica*. The dynamics of cercarial shedding was studied in experiment B. A single group of 2-mm-high *R. natalensis* was subjected twice to bimiracidial exposures as above (Table I).

After exposure, snails were reared in different types of covered aquaria with five snails per litre of permanently-oxygenated water. Water originated from a spring head located on calcareous soil so that the dissolved calcium

Shell height (mm)	Number of snails at exposure	Number (%) of surviving snails at day 28 p.e.	Number of infected snails (prevalence in %)
Snail dissection at day 28 p.e. (experiment A)			
Single exposures:			
1-2 mm	100	58 (58.0)	7 (12.0)
Double exposures			
1-2 mm	100	45 (45.0)	29 (64.4)
2.1-3 mm	50	31 (62.0)	18 (58.0)
3.1-4 mm.	50	37 (74.0)	7 (18.9)
4.1-5 mm.	50	44 (88.0)	1 (2.2)
5.1-6 mm	50	48 (96.0)	0
Cercarial shedding (experiment B)			
2 mm*	50	41 (82.0)	24 (58.5)

* Double exposed snail

Table I. – Survival at day 28 p.e. and prevalence of *F. hepatica* infection in seven groups of *R. natalensis*.

contents ranged from 60 to 73 mg/L. Pesticide-free lettuce was given *ad libitum* as food for snails. These aquaria were cleaned every week. They were placed in an air-conditioned room under the following conditions: a constant temperature of 24 ± 1 °C and a diurnal photoperiod of 12 hours with a 3,000-4,000 lux light intensity. In experiment A, all surviving snails were dissected on day 28 p.e. to detect an active infection. In experiment B, surviving snails were individually placed at day 30 p.e. in 35-mm diameter Petri dishes, with a piece of lettuce. The recipients are placed in the same air-conditioned room as the aquaria. Water and lettuce if necessary are changed every day. If metacercariae are present, they are counted and removed from dishes.

Parameters studied

The first two parameters studied in experiment A were snail survival at day 28 p.e. and the prevalence of infection with *F. hepatica* (calculated in relation with the number of dissected snails in each group). These percentages were compared using a χ^2 test (Stat-Itcf, 1988). In experiment B, snail survival at day 28 p.e. and the prevalence of infection (calculated in relation with the number of surviving snails at day 28) were also determined. The other parameters were the shell height of infected snails at day 45 p.e., the length of the prepatent period, that of the patent period, and the number of cercariae shed per infected snail. A χ^2 test and a one-way analysis of variance (Stat-Itcf, 1988) were used to establish levels of significance. The number of cercariae shed by each infected snail for each day of the patent period and the number of shedding waves were also determined. An autocorrelation test (Broom, 1979) was used to determine the existence of any periodicity in the daily distribution of cercariae throughout the patent period.

RESULTS

Characteristics of *F. hepatica* infection in snails

In the 1-2 mm snails (Table I) once subjected to bimiracidial exposures (experiment A), the survival of snails at day 28 p.e. was 58.0 % and the prevalence of infection was only 12.0 %. In snails twice exposed to *F. hepatica*, snail survival significantly raised (χ^2 = 29.85, P < 0.001) with increasing shell height at exposure while the prevalence of infection significantly diminished (χ^2 = 49.28, P < 0.001). Compared to the results noted in snails with single exposures, the prevalence was significantly greater (χ^2 = 30.57, P < 0.001) in the double exposed group of 1-2 mm snails whereas snail survival only showed an insignificant variation.
In snails studied for cercarial shedding (experiment B), snail survival at day 28 p.e. was 82.0 % while the prevalence of infection was 58.5 % (Table I). Of the 24 infected snails, 19 (46.3 %) have shed their cercariae and four snails died with no shedding. A single snail showed the presence of rediae when it was dissected after its death. The shell height of infected snails at day 45 p.e. was 10.3 ± 0.8 mm (data not shown).

Dynamics of cercarial shedding over time

In snails studied for cercarial shedding (experiment B), the length of the prepatent period was 66.5 ± 6.3 days while that of the patent period was 24.3 ± 11.0 days. Lastly, the number of *F. hepatica* cercariae counted after a cercarial shedding was 90.7 ± 10.2 (data not shown). Figure 1 shows the daily distribution of cercariae throughout the patent period. This number peaked at day 2 of the patent period (at 28.4 cercariae per snail) and progressively decreased afterwards up to day 14. Another peak (at 27.0 per snail) was also noted at

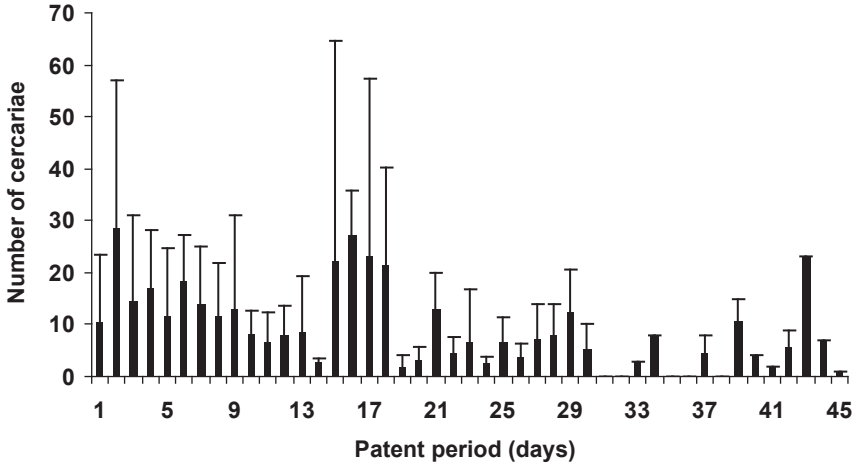

Fig. 1. – Number of *F. hepatica* cercariae shed by each cercaria-shedding snail for each day of the patent period.

day 16. Later, cercarial shedding was discontinuous over time with a few cercariae shed or no shedding up to the end of the patent period. Using the autocorrelation test, no infradian-type rhythm was noted in this daily distribution of cercariae.

In these cercaria-shedding snails, the number of shedding waves throughout the patent period ranged from 2 to 13. Five snails have shed their cercariae during four waves while four and three *R. natalensis* have released their cercariae during five and three waves, respectively. Six shedding waves were noted for two snails. Lastly, in the case of the five others, the respective numbers of shedding waves were 2, 7, 8, 9, and 13 (data not shown).

DISCUSSION

When the shell height of *R. natalensis* at exposure was increasing, snail survival at day 28 p.e. significantly augmented while the prevalence of infection decreased. These findings agreed with those reported by Dreyfuss *et al.* (2000) in another species of *Radix*, i.e. *R. balthica* (= *R. ovata*) when experimentally subjected to *F. hepatica* infection. The presence of infected snails without shedding or containing only free rediae of *F. hepatica* is more interesting to comment. Even if this fact was already noted in several snail species infected with *F. hepatica* (Vignoles *et al.*, 2002) or with *F. gigantica* (Shalaby *et al.*, 2004), the most plausible interpretation was to consider it to be an incomplete adaptation between the snail and its parasite (Boray, 1969). This first assumption is reinforced by the report by Lofty *et al.* (2002) according to which the introduction of *F. hepatica* in Egypt from imported cattle would be more recent than that of *F. gigantica*. However, another hypothesis based on a greater susceptibility of this Egyptian population of *R. natalensis* due to more frequent encounters of this snail with *F. hepatica* in the field, as demonstrated by Rondelaud (1993) for *G. truncatula*, cannot be excluded. As this Egyptian population of *R. natalensis* had already been used by Dar *et al.* (2003b, 2004) for experimental infections with sympatric isolates of *F. gigantica* (a single exposure with two miracidia for every snail), the results of these authors were compared to those reported for *F. hepatica* in the present study. Indeed, the environmental conditions of snail breeding were the same in both groups of experiments. Compared to the mean value (62.7 days) recorded for *F. gigantica*, the length of the prepatent period noted for *F. hepatica* was similar. In contrast, the length of the patent period was clearly lower (24.3 days instead of 37.8 for *F. gigantica)*. The same finding was also noted for the number of *F. hepatica* cercariae noted in double-exposed snails (90.7 cercariae/snail instead of 286.3/snail for *F. gigantica*). To explain this last difference, the most valid explanation is to relate this finding to the miracidial burden used for this above group (four miracidia per snail). Indeed, according to Rondelaud & Barthe (1987), the sporocyst of *F. hepatica* produced a higher number of rediae when the growth of *G. truncatula* was fast during the experiment. As the growth of *R. natalensis* during the 45 days of experiment was important (a mean of 10.3 mm), the production of this high redial burden would have limited the growth of these larvae within the snail and, as a consequence, the number of their cercariae owing to the quantity of nutrients available within the snail.

In spite of a mean patent period of 24.3 days noted for *F. hepatica*, all snails have shed their cercariae during 2-13 waves. As no snail shedding its cercariae during a single wave was noted in the present study, this finding underlined that natural encounter of this *Radix* population with the parasite would be frequent in the field. Indeed, according to Rondelaud (1993), most *G. truncatula* which lived along river banks and, as a consequence, had often rare or exceptional contacts with *F. hepatica*, often shed their cercariae during a single shedding wave when they were experimentally infected with this digenean. However, as there were several differences in the viability of *F. hepatica* metacercariae according to the susceptibility or the resistance of adult flukes to triclabendazole in the mammalian host (Walker *et al.*, 2006), it is necessary to determine if these cercariae produced by this Egyptian population of *R. natalensis* are viable in another definitive host.

The successful infection of this Egyptian population of *R. natalensis* with *F. hepatica* calls into question the specificity of this lymnaeid with this digenean. According to Bargues *et al.* (2001, 2003), Mas-Coma *et al.* (2009), every fluke (*F. hepatica* or *F. gigantica*) would have evolved in time in relation with its snail host (*Galba* and *Radix*, respectively) towards an increase of this specificity. This last point is more difficult to comment and two interpretations may be proposed. First, the positive infection of *F. hepatica* in *R. natalensis* might be due to the miracidial burden used to infect every double-exposed snail (a total of four miracidia). This was supported by the report by Lee *et al.* (1995) according to which the prevalence of *F. hepatica* infection was significantly increased when the number of miracidia used for each snail (from one to five) augmented. Secondly, this successful infection of *R. natalensis* might be due to the increased infectivity of French miracidia of *F. hepatica* used in the present study, as demonstrated by Dreyfuss *et al.* (2007). According to these authors, the infectivity of miracidia towards snails had increased in central France over the past years when they originated from eggs collected

from triclabendazole-treated cattle. Further investigations are still necessary to generalize this point by infecting other Egyptian and foreign populations of *R. natalensis* with sympatric or allopatric isolates of *F. hepatica*.

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