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Natural infection of *Fasciola hepatica* (Trematoda: Fasciolidae) in *Bulinus truncatus* (Gastropoda: Planorbidae) in northern Tunisia

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Abstract

Monthly samples of *Bulinus truncatus* were collected during a year from a cattle-breeding farm located in the region of Sejnane (North Tunisia) to detect natural infections with *Fasciola hepatica* and determine seasonal variations of the prevalence throughout a year. Of the 163 adult bulinids, larval forms of *F. hepatica* were found in 39% of snails. Two peaks in prevalence, the first in June and the second in October, were also noted. *Bulinus truncatus* can be added to the list of potential intermediate hosts of *F. hepatica*.

Some species of the family Lymnaeidae (Mollusca: Gastropoda) act as intermediate hosts in the life cycle of *Fasciola hepatica*. In Europe and in Africa, the main snail host is *Galba truncatula*. However, most of the other European species can also sustain the larval development of this parasite. This is the case with *Omphiscola glabra* if miracidial exposure occurs within the first 2 weeks of the snail's life (Boray, 1978) or if it is co-infected simultaneously with *F. hepatica* and *Paramphistomum daubneyi* (Abrous *et al.*, 1998, 2000; Dreyfuss *et al.*, 2003).

Besides lymnaeids, several freshwater pulmonates were also reported in the literature for their role as intermediate hosts. Successful infections of *Physa cubensis* with *F. hepatica* were reported by Viguera & Moreno (1938), but this result has never been verified in subsequent experiments (de Barros *et al.*, 2002). Cercaria-carrying rediae of *F. hepatica* have been found in experimental infections of juvenile *Bulinus truncatus* (Barthe & Rondelaud, 1986). Using experimental co-infections of *Anisus spirorbis* (= *Planorbis leucostoma*) with *F. hepatica* and *Paramphistomum daubneyi*, Abrous *et al.* (1998) reported successful infections of this snail with *F. hepatica*. Infection of *A. spirorbis* alone with *F. hepatica* was also shown in the field (Abrous *et al.*, 2000). The aim of the present note is to report natural infection of *B. truncatus* with *F. hepatica* in the region of Sejnane (North Tunisia), as this district is known to be a zone of endemic fasciolosis (Jemli *et al.*, 1991).

Unlike south-western Tunisia, for which *G. truncatula* has been reported as the single intermediate host of *F. hepatica* (Hammami & Ayadi, 1999; Hammami *et al.*, 2007), the snail host of this parasite had not been identified in the north. Parasitological investigations were thus carried out every month from April 2007 to March 2008 in a private farm located near the Ghar Ettin Lake in the Sejnane region (south-west of Bizerte town). The mean monthly temperature of this area was 16°C while the total rainfall recorded from April 2007 up to March 2008 was 704 mm. The ruminants living in this farm (mainly cattle) were frequently infected with *F. hepatica* and this natural infection was confirmed in 2007 by coproscopy (cattle: 50%; sheep: 47.8%) and by haemagglutination or electrosyneresis (cattle: 50%, sheep: 66.6%, goats: 27.7%). The sampling site (area, 1200 m^2) was a permanent pond fed only with run-off. The clayey soil was covered by 1-m deep stagnant water (Ca^{2+}: 134 mg/l; Cl^-: 73.9 mg/l) and the vegetation bordering this pond, with *Apium nodiflorum* and *Sonchus maritimus* as dominant species, was grazed by cattle and sheep. According to the farmer, cattle, sheep and

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goats were autochthonous and did not come from any exchange with other areas of Tunisia.

Bulinus truncatus was the single freshwater snail species living in the pond and no lymnaeid was found in the other meadows of this farm. A total of 163 snails, measuring more than 3 mm in height, were collected from this pond. After their collection, snails were transported to the lab and dissected under a stereo-microscope to detect any parasitic infection and identify species based on the morphology of larval forms. The identification of *F. hepatica* rediae was made using the criteria reported by Ollerenshaw & Graham (1986) and those by Dar *et al.* (2003), while cercariae of *F. hepatica* were recognized according to their morphology (Andrews, 1999).

Of the 163 *B. truncatus* collected throughout the study period, only *F. hepatica* was noted and no other infection or co-infection with another digenean was found. The presence of *F. hepatica* larval forms was noted in 65 snails (prevalence, 39.9%), and table 1 gives, for each month, the number of infected snails and the monthly prevalence of this natural infection. Two peaks, the first in June and the second in October, were observed. Free cercariae of *F. hepatica*, encysting or not after snail dissection, were found in most snails (data not shown). Shells of these infected bulinids ranged from 3 to 12 mm in height (data not shown).

The presence of *B. truncatus* as a natural intermediate host of *F. hepatica* in northern Tunisia is surprising as this species is usually known in North Africa to be the snail host of *Schistosoma* sp. (see review by Brown, 1994), of *Paramphistomum microbothrium* (Dinnik, 1965) or of *Echinostoma deserticum* (Kechemir *et al.*, 2002). The natural infection of this bulinid may be explained by accepting the hypothesis proposed by Kendall (1965, 1970) for European lymnaeids other than *G. truncatula*. According to this author, natural infections of several lymnaeids with *F. hepatica* occurred in countries where *G. truncatula* was absent. However, the height of infected *B. truncatus* (3–12 mm) suggests that infection of this bulinid with *F. hepatica* would be a long-established process and that adaptation between both partners would be complete.

Table 1. The prevalence of natural infection with *F. hepatica* in *B. truncatus* from April 2007 to March 2008.

Months	Number of snails investigated	Number of infected snails	Prevalence (%) of natural infection with *F. hepatica*
April	4	2	50
May	10	7	70
June	10	8	80
July	21	8	38
August	18	6	33.3
September	16	8	50
October	13	8	61.5
November	20	12	60
December	0	0	0
January	17	0	0
February	15	2	13.3
March	19	4	21
Total	163	65	39.9

These last assumptions are supported by the great infection rate (39.9%) noted in the present study, even if snail samples only concerned preadult and adult bulinids.

Two other results warrant comment. First, the two species of plants: *Apium nodiflorum* and *Sonchus maritimus*, found in the pond from the Sejnane region were also found in Gafsa oases, central Tunisia (Hammami *et al.*, 2007) and were suspected by these authors to be at the origin of local contamination of ruminants with *F. hepatica*. Indeed, the first species has been reported as a metacercaria-carrying plant in the natural watercress beds of the Limousin region, central France (Dreyfuss *et al.*, 2003). A larger survey on both plants in the Tunisian areas where fasciolosis occurs would be useful to determine their role in the local transmission of the disease. Second, the infection rate of *B. truncatus* (39.9%) found in the present study was greater than those noted for *G. truncatula* in Gafsa oases (19.2%: Hammami *et al.*, 2007) and in Tozeur oases (26%: Hammami & Ayadi, 1999). As this percentage was much higher than those reported by Ollerenshaw (1971) for *G. truncatula* in the UK (usually less than 2%) and by Mage *et al.* (2002) for the same species in central France (5.1%), the most valid explanation would be to relate this high value found in bulinids to the small number of snails (163 only) dissected in the present study.

The natural infection of *B. truncatus* with *F. hepatica* confirms the report by Barthe & Rondelaud (1986) and indicates that this bulinid is a potential intermediate host of this digenean. However, the mechanism by which *B. truncatus* became infected with *F. hepatica* in the natural environment remains to be shown. Three hypotheses were proposed to explain the larval development of *F. hepatica* in snails other than *G. truncatula*. The first involved the immaturity of the defence system in young snails at exposure, so that larval forms can develop (Boray, 1978). The second hypothesis might be an effect of facilitation in snails simultaneously co-infected by two digeneans, the first species penetrating the mollusc and favouring the development of the second parasite (Augot *et al.*, 1996). The last assumption may be the consequence of a particular aptitude that this bulinid population would have by sustaining frequent natural infections with another digenean (probably a species with two sporocyst stages in its life cycle), as demonstrated by Vignoles *et al.* (2007). According to these authors, populations of *G. truncatula*, known for their natural infections with a plagiorchiid, are better intermediate hosts for metacercarial production of *F. hepatica*. To verify these hypotheses, further experiments are necessary, subjecting young bulinids from this population and other bulinid communities living in the Sejnane region to single- or multiple-miracidium infections with *F. hepatica*, or to experimental co-infections with this digenean and another parasite such as *Paramphistomum* sp.

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