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Is *Radix peregra* a new intermediate host of *Fascioloides magna* (Trematoda) in Europe? Field and experimental evidence

(RNDr. thesis)

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České Budějovice, květen 2007

Horáčková, E., 2007: Is *Radix peregra* a new intermediate host of *Fascioloides magna* (Trematoda) in Europe? Field and experimental evidence. RNDr. Thesis, in English. Faculty of Biological Sciences, University of South Bohemia in České Budějovice, Czech Republic, 4pp.

Annotation

The ability of the giant liver fluke (*Fascioloides magna*) to expand the spectrum of its potential intermediate hosts, especially to use the aquatic snail *Radix peregra*, was investigated. Rediae and immature cercariae of the giant liver fluke, *Fascioloides magna*, a pathogenic and important parasite of game animals, were found in *Radix peregra* (Gastropoda, Lymnaeidae) from two localities in Western and Central Bohemia, Czech Republic. *R. peregra*, which is dominant over *Galba truncatula*, has never been reported as the natural intermediate host of *F. magna*. Its potential susceptibility to *F. magna* infection was confirmed experimentally. The present data indicate the capability of *F. magna* to expand the spectrum of its intermediate hosts and demonstrate the potential epizootiological importance of lymnaeid snails, other than *G. truncatula*, for transmission of the parasite in Europe.

České Budějovice, 2 May, 2007 …….

Author statement

I am the second author of the article " Is *Radix peregra* a new intermediate host of *Fascioloides magna* (Trematoda) in Europe? Field and experimental evidence", which has been publicated in the Acta Parasitologica, 2006, 51, 87-90. I contributed substantially to this paper.

Below, please find signatures of the first and other one autor confirming my contribution.

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DOI: 10.2478/s11686-006-0013-9

Acta Parasitologica, 2006, 51(2), 87–90; ISSN 1230-2821

VERSITA

Is *Radix peregra* a new intermediate host of *Fascioloides magna* (Trematoda) in Europe? Field and experimental evidence

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Abstract

Rediae and immature cercariae of the giant liver fluke, *Fascioloides magna*, a pathogenic and important parasite of game animals, were found in 6 out of 7,277 *Radix peregra* (Gastropoda, Lymnaeidae) from two localities in Western and Central Bohemia, Czech Republic. In one of these localities a relatively high infection of *Galba truncatula* (26 out of 76) with mature cercariae of *F. magna* was detected. *R. peregra*, which is dominant over *Galba truncatula*, has never been reported as the natural intermediate host of *F. magna*. Its potential susceptibility to *F. magna* infection was confirmed experimentally with 67 snails of *R. peregra* (out of 371) infected. The present data indicate the capability of *F. magna* to expand the spectrum of its intermediate hosts and demonstrate the potential epizootiological importance of lymnaeid snails, other than *G. truncatula*, for transmission of the parasite in Europe.

Key words

Fascioloides magna, Trematoda, *Lymnaea* (*Galba*) *truncatula*, *Radix peregra*, snails, life cycle, epizootiology

Introduction

The giant liver fluke, *Fascioloides magna* (Bassi, 1875), introduced to Europe in the 19th century from North America, is an important parasite of wild and domestic ruminants (Swales 1935, Erhardová 1961, Erhardová-Kotrlá 1971). Red deer (*Cervus elaphus*), fallow deer (*Dama dama*) and roe deer (*Capreolus capreolus*) are common European definitive hosts of the fluke (Erhardová 1961, Erhardová-Kotrlá 1971). In Europe, initially the fluke was found in Italy, Czech Republic, Germany and Poland (Bassi 1875, Ullrich 1930, Salomon 1932, Ślusarski 1955, Erhardová 1961, Balbo *et al*. 1989). Recently it spread to Austria, Croatia, Hungary and Slovakia (Pfeiffer 1983; Majoros and Sztojkov 1994; Rajský *et al*. 1994, 2002; Špakulová *et al*. 1997; Marinculić *et al*. 2002).

At least 10 species of lymnaeid snails are known as natural and experimental intermediate hosts of *F. magna* in North America (Dunkel *et al*. 1996), whereas only *Galba truncatula* (Müll.) (Gastropoda, Lymnaeidae) has been confirmed as the natural intermediate host in Europe (Erhardová-Kotrlá 1968, 1971). In the present study, the natural occurrence of larval stages of *F. magna* in another lymnaeid snail, *Radix peregra* (Müll.), is reported for the first time and its susceptibility to miracidia of *F. magna* under laboratory conditions is tested.

Materials and methods

From 2002 to 2005 *Galba truncatula* and *Radix peregra* snails were collected repeatedly in 9 localities in Western, South and Central Bohemia, Czech Republic, where *Fascioloides magna* infections of game animals were reported. The enzootic areas of *F. magna* were confirmed by coprological and post mortem examinations of game. The number of *R. peregra* snails sampled in individual localities is summarized in Table

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I. Snails were separated into vials with water; shedding of cercariae was stimulated by light and heat over night. Thereafter, all snails were dissected to detect larval trematodes other than mature cercariae. The trematodes were examined alive under light microscope and stereomicroscope; for measurements they were fixed with hot formaldehyde solution (4%).

two localities in Western and Central Bohemia (Table I). Mature cercariae were also detected in 26 of 76 *Galba truncatula* examined (prevalence 34%) from one of the localities (Sedliště, near Plzeň) where *R. peregra* was infected with *F. magna*. Cercariae from *R. peregra* corresponded in their morphology to those of *F. magna* observed in the common natural inter-

Table I. Survey of localities and numbers of *Radix peregra* snails examined, infected and the prevalence (%)

Locality (District)	No. examined	No. infected	%
Sedliště, enclosure (Plzeň)	2,436	3	0.12
Obecnice, military area, (Příbram)	108	3	2.78
Jince, military area (Příbram)	62	–	–
Strašice, military area (Příbram)	52	–	–
Dobříš, enclosure "Aglaia" (Příbram)	477	–	–
Blatná (Strakonice)	18	–	–
Poněšice (České Budějovice)	3,513	–	–
Boletice, military area (Český Krumlov)	529	–	–
Byňov, Jakule (Nové Hrady)	82	–	–
Total	7,277	6	0.08

Eggs for experiments were isolated from the liver of fallow deer from the enclosure in Sedliště (Plzeň), Western Bohemia. For infections, two groups of laboratory-reared *Radix peregra* (groups 1 and 2) and *Lymnaea stagnalis* (4) were used as well as *G. truncatula* (5) originating from the Czech Republic; another group of *R. peregra* (3) from Central France, Haute Vienne, was used (Table II). Eggs were incubated in tap water in Petri dishes under laboratory temperature (23°C) for at least 32 days when miracidia started to hatch; water was changed every other day. For infections the snails were placed individually in Petri dishes with water and exposed for 1–3 hours to miracidia (Table II). After exposure the snails were maintained at 17°C in aquaria and afterwards examined as stated above.

Results

Developmental stages of *Fascioloides magna* (rediae and immature cercariae) were found in six *Radix peregra* snails from

mediate host, *G. truncatula*, from the same locality (Sedliště near Plzeň – see Hirtová *et al*. 2003) and those reported by Erhardová-Kotrlá (1971). The cercariae were identical in the body shape, body surface, position of the inner organs, the presence of suckers of nearly equal size, oval pharynx and the intestinal bifurcation situated at the same position, the absence of a prepharynx and the shape of the excretory bladder. Because of immaturity, the cercariae from *R. peregra* were not able to encyst. Their tail was shorter (only slightly longer than their body) than in the cercariae of *F. magna* from *G. truncatula*. Their cystogenous glands and cells with bacillary particles were less developed and less numerous, and refractile granules in the excretory ducts were smaller, and therefore the body was more transparent (Fig. 1A-C).

In experiments, fully formed (mature) cercariae, as well as mother and daughter rediae, were obtained from 67 *R. peregra* (Fig. 1D), which demonstrates the susceptibility of this snail to *F. magna* infection. The first mother rediae were recovered 35 days post infection, and cercariae were found 119 days p.i. Infection was successful only in small *R. peregra* (shell height

Table II. Survey of experimental infections of snails with miracidia of *Fascioloides magna*

Snail species	Shell height (mm)	Dose	Dissected (days p.i.)	exposed	No. snails survived	infected	Rediae	Cercariae
R. peregra	1.5–15	10–40	54, 70, 116	99	91	–	–	–
R. peregra	1–8	3–5	35–131	222	152	63	60	3
R. peregra	3–6	3	65	50	35	4	4	–
L. stagnalis	8–16	20	54, 70, 116	20	16	–	–	–
G. truncatula	3–4	2	60	30	15	15	10	5

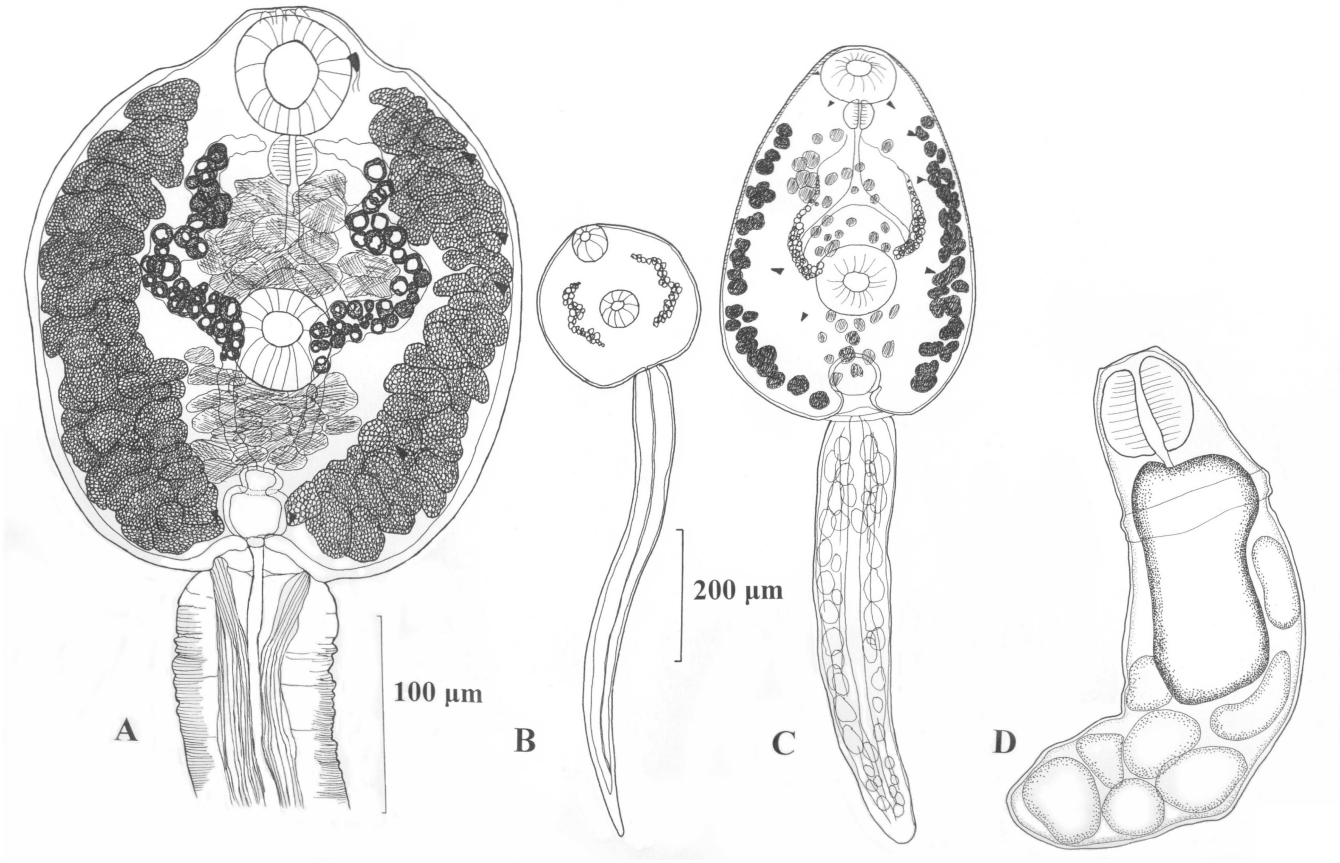

Fig. 1A-D. *Fascioloides magna*: **A** and **B** – mature cercaria from naturally infected *Galba truncatula* (A – body, B – total view); **C** – immature cercaria from naturally infected *Radix peregra*; **D** – redia of *F. magna* from experimentally infected *R. peregra*

1–8 mm) and *G. truncatula* snails, whereas larger *R. peregra* and all *Lymnaea stagnalis* snails remained uninfected (Table II), despite being exposed to a higher dose of miracidia.

The cercariae from the experiment are morphologically the same as the cercariae from natural infections of *R. peregra* and *G. truncatula*. The rediae from *R. peregra*, from nature and from the experiments, had two ambulatory buds, a collar (mother rediae), a well developed pharynx and a short sac-like gut with a greenish content (Fig. 1D), which correspond to the morphology of rediae from *G. truncatula* as reported by Erhardová-Kotrlá (1971).

Discussion

Species identification of larval stages found in *Radix peregra* was based on morphological characteristics of the cercariae and rediae from nature and in experiments (Fig. 1) and was compared with the description provided by Erhardová-Kotrlá (1971). Moreover, no adults of *Fasciola hepatica* were recovered from livers of game animals in the localities examined (Hirtová *et al*. 2003, Novobilský *et al*. 2005).

The present data, based on field observations and experimental infections, indicate the capability of *R. peregra* to serve as the intermediate host of *F. magna*. The occurrence of the parasite in *R. peregra* in Western Bohemia (Sedliště, Plzeň) can be explained by an extremely high parasite load in fallow deer that shed a high quantity of eggs into a limited space of the game enclosure (40 ha). Because the population density of *R. peregra* was very high in this locality, a close contact of miracidia and snails over a long period was ensured. Also the presence of *G. truncatula* with a relatively high rate of infection helped to sustain the infection. However, the other locality in Obecnice (Brdy) is much larger, is not enclosed by any fence, and the game animals can migrate over vast areas. Despite this fact, the same number of *R. peregra* infected with *F. magna* was observed there.

Results of experimental infections demonstrated the capability of *F. magna* miracidia to infect *R. peregra* snails, transform to sporocysts and to both generations of rediae and cercariae. This situation differs from the experiments of Erhardová-Kotrlá (1968), who reported that the parasite developed only to the stage of mother rediae in *R. peregra* and *R. peregra ovata*. Complete development of *F. magna* cercariae in exper-

imentally infected *R. peregra* snails, together with natural findings in the present study, may indicate that the parasite undergoes a process of adaptation to other intermediate hosts.

It is apparent that further field and laboratory studies should be performed in order to trace the ability of *F. magna* to infect a broader spectrum of intermediate hosts in Europe. Apart from *R. peregra*, also *Lymnaea* (*Stagnicola*) *palustris* and *Omphiscola glabra* were reported as experimental intermediate hosts and fully formed, encysting cercariae were obtained (Chroustová 1979, Rondelaud *et al*. 2006). Therefore, future studies on the biology and epizootiology of *F. magna* should focus on the role of lymnaeid snails other than *G. truncatula* as snail intermediate hosts in Europe. Special attention should be given to the actual role of *R. peregra* in the transmission of *F. magna* because this snail is very common in Central Europe and dominates over *G. truncatula* in the areas with occurrence of *F. magna*.

Acknowledgements. This study was supported by the Ministry of Education, Youth and Sports of the Czech Republic (projects Nos 1054/2004 and J16/98: 161700001; LC522), Grant Agency of the Academy of Sciences of the Czech Republic (A6022404), Grant Agency of the Czech Republic (524/03/H133), Institute of Parasitology, ASCR (Z60220518) and the Faculty of Biological Sciences, University of South Bohemia. The authors are deeply indebted to V. Kolář and J. Machatý for their help in the field, K. Chroust, O. Ditrich, Z. Fric and B. Koudela for valuable advice and M. Borovková, B. Škoríková and L. Kablásková for their help with mollusc sampling.

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(Accepted February 15, 2006)