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Lymnaea fuscus (Pfeiffer, 1821) as a potential intermediate host of *Fascioloides magna* in Europe

Adam Novobilský [a,*], Martin Kašný [b], Jan Pankrác [b], Daniel Rondelaud [c], Annie Engström [a], Johan Höglund [a]

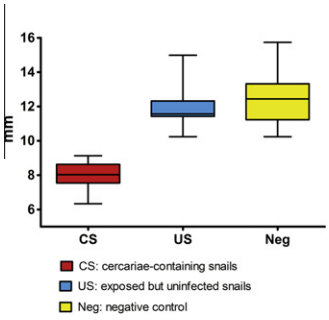
[a] Swedish University of Agricultural Sciences (SLU), Department of Biomedical Sciences and Veterinary Public Health, Section for Parasitology, 75007 Uppsala, Sweden
[b] Charles University in Prague, Faculty of Science, Department of Parasitology, Viničná 7, 128 44 Prague 2, Czech Republic
[c] INSERM U 1094, Faculty of Medicine and Faculty of Pharmacy, 2 rue du Docteur Raymond Marcland, 87025 Limoges, France

HIGHLIGHTS

- We experimentally infected *Lymnaea* (*Stagnicola*) *fuscus* with *Fascioloides magna*.
- Complete larval development of *F. magna* was observed in *L. fuscus*.
- Limited cercarial production and age resistance of snail to *F. magna* was noted.
- *L. fuscus* should be added to the list of *F. magna* European intermediate hosts.

GRAPHICAL ABSTRACT

The effect of *F. magna* infection on growth of *Lymnaea fuscus* (comparison of shell height at day 85 p.e.).



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ABSTRACT

Experimental infections of two different populations of *Lymnaea fuscus* in France and Sweden, with a Czech isolate of *Fascioloides magna* were carried out to determine if this lymnaeid species enables parasite larval development. Species identification of both snail populations was performed using the morphology of the copulatory organ, and also confirmed by sequencing of the internal transcribed spacer 2 (ITS2) region of the snail genomic rDNA. Only juvenile snails measuring less than 3 mm (1–3 weeks of age) were successfully infected (the viable cercariae were recorded) and infection prevalence decreased with age, as documented by increased shell height. In both French and Swedish *L. fuscus* populations, prevalence ranged between 1.1% and 58.8%. The mean number of metacercariae obtained from cercariae-shedding snails was 13.7 (±11.4), while the total cercarial production noted in snails dissected at day 85 post-exposure was 147.5 (±56.6). Compared to uninfected control snails, we observed reduced growth of infected snails. Despite age-related resistance of snail to the parasite, and limited cercarial production in these experimentally infected snails, *F. magna* was still able to complete larval development in *L. fuscus*.

1. Introduction

The giant liver fluke *Fascioloides magna* is a pathogenic parasite infecting a variety of wild and domestic ruminants, primarily in North America but since the second half of 19th century also in Europe (Swales, 1935; Erhardová-Kotrlá, 1971). Today, fascioloidosis in wild cervids occurs in many places of central Europe, including Austria (Ursprung et al., 2006), Croatia (Marinculić et al., 2002),

* Corresponding author. Address: Swedish University of Agricultural Sciences, Department of Biomedical Sciences and Veterinary Public Health, Section for Parasitology, Box 7028, 75007 Uppsala, Sweden. Fax: +46 18673334.
E-mail addresses: anovobilsky@yahoo.com, Adam.Novobilsky@slu.se (A. Novobilský).

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the Czech Republic (Novobilský et al., 2007; Kašný et al., 2012), Hungary (Majoros and Sztojkov, 1994), Italy (Balbo et al., 1989), and Slovakia (Špakulová et al., 2003). During recent decades the geographical range of *F. magna* distribution has increased, and several other European countries than those mentioned above are clearly at the risk of its introduction. For instance, *F. magna* is spreading at the Danube river floodplain forests (Špakulová et al., 2003), and was recently confirmed by findings in fallow deer (*Dama dama*) in Serbia (Marinkovic and Nesic, 2008).

The snail *Galba truncatula* is considered to be the most common natural intermediate host of *F. magna* in Europe (Erhardová-Kotrlá, 1971; Špakulová et al., 2003; Vignoles et al., 2006). However, other European lymnaeid snails, such as *Radix peregra* (Faltýnková et al., 2006; Kašný et al., 2012) and *Lymnaea* (*Omphiscola*) *glabra* (Rondelaud et al., 2006), have also been reported as susceptible to *F. magna* infection. Furthermore, European stagnicoles (e.g. *Lymnaea palustris*) are also the subject of interest, due to their potential ability to serve as intermediate hosts of several fasciolid species (Bargues et al., 2001).

Recently, the phylogenetic relationships within the family Lymnaeidae were investigated (Correa et al., 2010). According to this taxonomic revision, all European species in the genera *Lymnaea*, *Stagnicola* and *Omphiscola* belong to the same clade, and should be named *Lymnaea* according to the principle of priority in the International Code of Zoological Nomenclature (Correa et al., 2010). Thus, European *Stagnicola* spp. have regained their old names and recently been renamed to *Lymnaea*. This genus currently contains three species: *L. palustris*, *L. fuscus*, and *L. corvus* (this nomenclature will be used in our text). Several studies on susceptibility of stagnicolid species to *F. magna* have already been reported. In North America, both *S. palustris* and *S. palustris nuttuliana* were shown as suitable intermediate hosts of *F. magna* (Swales, 1935; Griffiths, 1962). However, *L. palustris* has also been synonymised with Nearctic stagnicolid species such as *Hinkleyia* (*Stagnicola*) *elodes* (Burch, 1989) in the past. Despite anatomical and ecological similarities between North American stagnicolids (currently unified into the genus *Hinkleyia*) and Palaearctic stagnicolids (i.e. genus *Lymnaea*), are phylogenetically separated from each other according to the latest taxonomical revision (Remigio and Blair, 1997; Correa et al., 2010).

L. fuscus (Pfeiffer, 1821) is a common Palaearctic snail which has been synonymised with *L. palustris* in the past (Jackiewicz, 2000). It is common in freshwater habitats in Europe (Glöer and Meier-Brook, 1998), including countries with enzootic localities of *F. magna* infection such as the Czech Republic (Beran, 2008) and Croatia (Beran, 2011). In some countries such as in France and Sweden, *L. fuscus* is even more abundant than *L. palustris* and *L. corvus* (Nilsson et al., 1998; Dreyfuss et al., 2000). Until now, however, there is very limited information available about the ability of *L. fuscus* to act as an intermediate host for trematodes. The above described taxonomical problems with *L. palustris* have most likely contributed to this confusion. Dreyfuss et al. (2000) reported that *Fasciola hepatica* can complete its larval development in experimentally infected juvenile *L. fuscus*. In Europe, Czech populations of *L. palustris* were successfully infected with *F. magna* under laboratory conditions (Chroustová, 1979), but to our knowledge information about the susceptibility of *L. fuscus* and *L. corvus* to *F. magna* is still lacking.

Owing to the potential risk of *F. magna* spreading in Europe by natural migration of deer or accidental introduction of animals by humans, it is meaningful to determine all possible *F. magna* intermediate hosts, in order to assess their epidemiological significance. Since *L. fuscus* represents a lymnaeid snail species sharing similar habitats with *G. truncatula* (Erhardová-Kotrlá, 1971) and as it also occurs in endemic areas of fascioloidosis in Europe (Špakulová et al., 2003), the aim of the current study was to determine the susceptibility of two populations of *L. fuscus* to *F. magna* under experimental conditions.

2. Materials and methods

2.1. Snails and parasite

Two geographically separated populations of *L. fuscus* were used in the present study. The first population (F) originated from a small pond (46° 35′ 38″ N, 1° 27′10″ E) near the farm "Les Marauts" located in the commune of Thenay, department of Indre, central France. A total of 750 juvenile snails, measuring 1–4 mm in height, were collected from this site. The second population (S) was collected in 2010 from a drainage ditch (58° 5′ 13″ N, 12° 13′ 8″ E) at a sheep farm near Lilla Edet, Västra Götalands County, Sweden. As this population was maintained in the laboratory (Department of Biomedical Sciences and Veterinary Public Health, Swedish University of Agricultural Sciences, Uppsala, Sweden), a total of 200 juvenile snails (2–5 mm) belonging to the F2 generation was used for this study.

Unembryonated eggs of *F. magna* were recovered in October 2010 from livers of red deer (*Cervus elaphus*) in an enzootic area of fascioloidosis in Krivoklatsko district, Czech Republic (Novobilský et al., 2007). These eggs were washed several times with 0.1 M pH 7 phosphate buffer saline (PBS), and stored in spring water at 4 °C until needed. Prior to the experiment, eggs were incubated in Petri dishes for 12 days at 24 °C in the dark (Ollerenshaw, 1971) and then stimulated to hatch by placing them under intensive light for 30 min.

2.2. Snail species identification

Snails of both populations (*F* and *S*) were identified on the basis of shell morphology and the length of their reproductive organs according to the keys by Glöer and Meier-Brook (1998) and Jackiewicz (2000). Furthermore, molecular taxonomic identification of snail species was based on amplification and sequencing of the internal transcribed spacer 2 (ITS2) region of the ribosomal rDNA, as follows.

Genomic DNA from soft tissues (2 adults per population) was extracted using the DNeasy Blood and Tissue Kit (Qiagen, Germany) according to the manufacturer's instructions. A region of ITS2 of the snail rDNA was then amplified by PCR according to Correa et al. (2010), using primers designed by Almeyda-Artigas et al. (2000): forward 5′-TTCTATGCTTAAATTCAGGGG-3′ and reverse 5′-TGTGTCGATGAAGAACGCAG-3′. PCR products were subsequently separated on 1.5% agarose gel. Five microliters of PCR product were purified by mixing with 0.5 μl exonuclease I and 1 μl shrimp alkaline phosphatase (Fermentas, USA). The mix was incubated at 37 °C for 15 min and the reaction was stopped by incubation at 85 °C (15 min). Purified PCR products were processed by using the BigDye Terminator v.3.1 cycle sequencing kit (Applied Biosystems, USA) and finally sequenced on an ABI 3100 Genetic Analyzer BDT 3.1 (Applied Biosystems, USA). The sequences obtained were edited with CLC Main Workbench version 5.6.1 and aligned with sequences from the NCBI database using the BLAST tool accessed in February 2012.

2.3. Experimental infections of snails

Table 1 explains the purpose of each experiment, the characteristics of the snails and the number of *F. magna* miracidia used at each exposure. In experiment (A), three groups of juvenile *L. fuscus* differing in shell height (1.0–2.0, > 2.0–3.0, or >3.0–4.0 mm) were exposed to miracidia to study patterns of cercarial shedding, while