Polish Journal of Veterinary Sciences Vol. 17, No. 3 (2014), 523–525

DOI 10.2478/pjvs-2014-0077

Short communication

Molecular identification of *Fascioloides magna* (Bassi, 1875) from red deer from South-Western Poland (Lower Silesian Wilderness) on the basis of internal transcribed spacer 2 (ITS-2)

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Abstract

The study was conducted in 2012-2013 on 75 fecal samples of red deer from the Lower Silesian Wilderness which were examined to determine the prevalence of *Fascioloides magna* in the game population. Finding liver fluke eggs in a single sample which were larger in size than *Fasciola hepatica* eggs indicated that further molecular analysis was necessarily. The partial sequence (116 bp long) of ITS-2 of the investigated eggs was identical to the sequences of *F. magna* from red deer (*Cervus elaphus*) (GenBank, EF534993; GenBank, EF534992) and from wapiti deer (*Cervus elaphus canadensis*) (GenBank, EF534994) from Slovakia, as well as from fallow deer (*Dama dama*) from the USA (GenBank, EF051080). This is the first molecular confirmation of the occurrence of *F. magna* in Poland.

Key words: *Fascioloides magna*, PCR, ITS-2, red deer, *Cervus elaphus,* Poland

Introduction

Fascioloides magna is a liver trematode of high pathogenic potential for wild and domestic ruminants. The species originates from North America where it has found several species of definitive hosts among wild cervids such as wapiti deer (*Cervus elaphus nelsoni*), white-tailed deer (*Odocoileus virginianus*), caribou (*Rangifer tarandus*), mule deer (*Odocoileus hemionus hemionus*) and black-tailed deer (*Odocoileus hemionus columbianus*) (Pybus 2001). However, the parasite spread to Europe in the 19th century due to the introduction of North American cervids, and was able to find new species of definitive hosts under European conditions, i.e., red deer (*Cervus elaphus*), fallow deer (*Dama dama*), as well as roe deer (*Capreolus capreolus*) (Swales 1935). The first European case of infection was detected in Italy (Bassi 1875). Recently, intensive spread of this parasitosis has been observed throughout Europe. So far it

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Table 1. Origin of sequences of internal transcribed spacer region 2 of *Fascioloides magna* used in the alignment in Fig. 1.

Genotype	GenBank No.	Host	References
Poland	–	*Cervus elaphus*	Present study
Slovakia1	EF534993.1	*Cervus elaphus*	Kralova-Hromadova et al. 2008
Slovakia2	EF534994.1	*Cervus elaphus canadensis*	Kralova-Hromadova et al. 2008
Slovakia3	EF534992.1	*Cervus elaphus*	Kralova-Hromadova et al. 2008
USA	EF051080.1	*Dama dama*	Unpublished

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                    *        20         *        40         *
Poland    : ggcgatcccctagtcggcacatttacgatttctgggatgatcccatacca :  50
Slovakia1 : GGCGATCCCCTAGTCGGCACATTTACGATTTCTGGGATGATCCCATACCA :  50
Slovakia2 : GGCGATCCCCTAGTCGGCACATTTACGATTTCTGGGATGATCCCATACCA :  50
Slovakia3 : GGCGATCCCCTAGTCGGCACATTTACGATTTCTGGGATGATCCCATACCA :  50
USA       : GGCGATCCCCTAGTCGGCACATTTACGATTTCTGGGATGATCCCATACCA :  50

                   60         *        80         *        100
Poland    : ggcacgttccactactgtcgctttatcgtcggtttgatgctaggcttggt : 100
Slovakia1 : GGCACGTTCCACTACTGTCGCTTTATCGTCGGTTTGATGCTAGGCTTGGT : 100
Slovakia2 : GGCACGTTCCACTACTGTCGCTTTATCGTCGGTTTGATGCTAGGCTTGGT : 100
Slovakia3 : GGCACGTTCCACTACTGTCGCTTTATCGTCGGTTTGATGCTAGGCTTGGT : 100
USA       : GGCACGTTCCACTACTGTCGCTTTATCGTCGGTTTGATGCTAGGCTTGGT : 100

                    *
Poland    : catgtatctgatgcta : 116
Slovakia1 : CATGTATCTGATGCTA : 116
Slovakia2 : CATGTATCTGATGCTA : 116
Slovakia3 : CATGTATCTGATGCTA : 116
USA       : CATGTATCTGATGCTA : 116
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Fig. 1. Alignment of *Fascioloides magna* ITS-2 rRNA sequences; *Poland* – sequence obtained in this study, *Slovakia1* (GenBank, EF534993), and *Slovakia3* (GenBank, EF534992) sequences from *Cervus elaphus* isolates from Slovakia; *Slovakia2* (GenBank, EF534994) – isolate from *Cervus elaphus canadensis* from Slovakia; *USA* (GenBank, EF051080) – isolate from *Dama dama* from USA.

has been reported from Germany, the Czech Republic, Austria, Croatia, Hungary, and the Slovak Republic (Kasny et al. 2012), as well as from Poland, where it was first noted in 1955 (Ślusarski). The aim of the study was molecular verification of *F. magna* infection of red deer in Poland.

Materials and Methods

Seventy-five fresh fecal samples were collected from red deer in 2012-2013 from the southwest part of Poland (Lower Silesian Wilderness, Ruszów Forestry Management). Three grams of each sample were examined for trematode eggs using the sedimentation method. Eggs were counted in Petri dishes under a microscope (40× magnification). Measurements of 30 eggs were done with the use of a light microscope (Jenaval) at 125× magnification. All dimensions of eggs are given in µm.

The genomic DNA of 50 eggs of *F. magna* (fixed in 70% ethanol) was isolated with DNeasy Blood & Tissue Kit (Qiagen) according to the enclosed protocol. The ITS-2 genes of *F. magna* were amplified with the use of one pair of specific primers: forward FM_ITS2_SPEC_F (5'-ACCAGTTATCGTTG TGTTG-3') and reverse FM_ITS2_SPEC_R (5'-CCGTCTTTAAACAACAG-3') to achieve a 152 bp long product (Králová-Hromadová et al., 2008). A PCR reaction was conducted in a total volume of 25 µl and contained: 0.2 mM each of deoxynucleotide triphosphate (Novazym), 20 mM of each primer,

1 U of HiFi *Taq* DNA Polimerase (Novazym), *Taq* DNA Polimerase Buffer (×10) (700 mM Tris-HCl, pH 8.6, 166 mM $(NH_4)_4SO_4$, 25 mM $MgCl_2$), and 1 µl of DNA template. Amplification was conducted in a Techne TC-512 Thermal Cycler in accordance with Kašný et al. (2012). The product was purified with Nucleospin Gel and PCR Clean-up kit (Macherey-Nagel) and prepared for sequencing which took place in Genomed S. A. The final alignment was performed using ContigExpress software. The obtained sequence was aligned and compared with the sequences of ITS-2 of *Fascioloides magna* that were available in GenBank (Table 1) using GenDoc-Multiple Sequence Alignment Editor software.

Results and Discussion

Fifty-two trematode eggs were detected in a single deer-derived fecal sample. They were yellowish, carrying a tiny operculum and were significantly larger than *Fasciola hepatica* eggs (138.4-167.5 × 84.5-104.3; mean 84.5 × 95.1, respectively). The partial sequence of ITS-2 (116 bp long) obtained in this study was identical to the sequences of the same gene fragment of *Fascioloides magna* isolated from red deer from Slovakia and from fallow deer from the USA (Fig. 1; Table 1). The results presented above confirm the occurrence of *F. magna* in Poland.

F. magna was first detected in Poland in 1953 in the liver of a red deer aged 6 years, which was hunted in the Lower Silesia Forest, near Bolesławiec (Ślusarski 1955). *F. magna* was proved to be highly pathogenic also for domestic ruminants, as observed both in experimental and natural infections (Foreyt and Leathers 1980, Novobilský et al. 2007). Additional investigations including occurrence and dispersion of the trematode in red deer as well as in domestic animals in Poland, are required.

Acknowledgments

The authors would like to express their gratitude to the Forest District Manager of the Ruszów Forest District, Janusz Kobielski, M. Sc. and Dr. Dorota Merta of the Pedagogical University of Cracow for their help in collecting the materials.

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