

Natural infection with *Fasciola hepatica* (Linnaeus, 1758) in the European bison (*Bison bonasus*) in Białowieża National Park, Poland

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Summary

The aim of our study was to determine prevalence of appearance of *Fasciola hepatica* L. among European bison *Bison bonasus* selected in Białowieża National Park, Poland from 2001 to 2006. The analyzed ITS-1 and ITS-2 sequences of nuclear DNA of *Fasciola hepatica* have a length of 433bp and an AT content of 47.8 % and a length of 365 bp and an AT content of 51.2 % respectively. Among 178 bison liver fluke was found in liver of 63 (35.3 %) individuals including as follow: in 39 (21.9 %) adults, 11 (6.1 %) juveniles and 13 (7.3 %) calves. Results of researches are as follow: prevalence of infection of *Fasciola hepatica* among bison in Białowieża National Park between 2001 – 2006 oscillated about 35.3%. The most extensive invasion of fascioliasis was observed in adult animals (21.9 %). We pay attention that between infected animals were calves 13 (7.3 %). Conclusions: The obtained results referring to infection of bison within the Białowieża National Park, Poland of *F. hepatica* are very interesting. The epidemiological importance of these finding is evident, demonstrating the fascioliasis expansion in restricted or protected areas, as National Parks such as Białowieża National Park, habitat of this wild host, and where no domestic animals are present.

Keywords: *Fasciola hepatica*; nuclear DNA; *Bison bonasus*; Białowieża National Park; Poland

Introduction

Fascioliasis caused by liver fluke *Fasciola hepatica* is a worldwide parasitic disease common in ruminants, especially cattle, buffaloes, sheep, goats, and swine. It may, however, affect bison, suckers, black rat, ostriches, hares and humans (Mas-Coma *et al.*, 1998; 1999; Valero *et al.*, 1999). Once ingested, parasites migrate through the liver parenchyma to reach the bile ducts. In ruminants, the liver

is damaged and condemned, and the subclinical and chronic disease usually results in decreased production of meat, milk and wool, secondary bacterial infections, fertility problems, and great expenses with anthelmintics (Marques & Scroferneker, 2003; Faria *et al.*, 2005). *Fasciola hepatica* has a cosmopolitan distribution. It can be assumed that liver fluke is European origin, with the snail *Limnaea truncatula* as the original intermediate host. Some authors suggest the potential of this wild host in the fascioliasis transmission (Bargues *et al.*, 2001, 2012; Khoubbane *et al.*, 2004; Mas-Coma *et al.*, 2009). The liver fluke *Fasciola hepatica* plays an important role as a parasite of wild and farm animals. Humans become infected after eating aquatic plants on which encysted metacercarias are present or by drinking contaminated water. Over the period 1970 – 1990 human cases were reported in 42 countries, and the World Health Organization now recognizes fascioliasis as a dangerous disease in humans. The *Fasciola hepatica* is responsible for human fascioliasis, a major parasitic disease widely distributed throughout the world affecting globally several million of people, while a further 180 million are at risk (Esteban *et al.*, 2002; Saba *et al.*, 2004). In hypo- to hyper-endemic areas of Central and South America, Europe, Africa and Asia, human fascioliasis presents a range of epidemiological characteristics related to a wide diversity of environments (Mas-Coma 2005). Circle of hosts of this liver fluke is coming wider every year also in East Europe.

For research a population of wild European bison *Bison bonasus* living in Białowieża National Park, Poland were chosen.

The aim of our study was to determine prevalence of appearance of liver fluke *Fasciola hepatica* L. among European bison *Bison bonasus* selected in Białowieża National Park in 2001 – 2006.

Tab. 1. Prevalence of infection with *Fasciola hepatica* by age - group in Bison bonasus in Białowieża National Park, Poland, 2001 – 2006

Period	Number of examined animals	Percentage of examined animals	Number of infected animals							
			Individuals	%	Adults	%	Juveniles	%	Calves	%
Dec 2001 – Mar 2002	35	19.6	6	17.1	2	5.7	0	0	4	11.4
Dec 2002 – Mar 2003	40	22.4	15	37.5	12	30	2	5	1	2.5
Oct 2003 – Mar 2004	35	19.6	11	31.4	8	22.8	3	8.5	0	0
Dec 2004 – Apr 2005	34	19.1	9	26.4	9	26.4	0	0	0	0
Nov 2005 – Mar 2006	34	19.1	22	64.7	8	23.5	6	17.6	8	23.5
In average	35.60		12.60		7.80		2.20		2.60	
Total number	178	100	63	35.3	39	21.9	11	6.1	13	7.3

Material and methods

Prevalence of invasion of the adult liver fluke *Fasciola hepatica* (Animalia, Trematoda, Digenea, Fasciolidae, *Fasciola, Fasciola hepatica* Linnaeus, 1758) in common ducts bile of liver European bison *Bison bonasus* (białowieski) in Białowieża National Park, Poland was investigated in 2001 – 2006. Within Polish area of Białowieża National Park the population of bison in herd consists about 351 animals and within Belorussian area about 275 animals (Krasinska & Krasinski, 2004).

Samples of common ducts bile of liver of (necropsies) bison from 3 months-old to 17 years old killed during selection match were received post-mortem (autopsy). The method directly dissected the common ducts bile of liver were examined where macroscopic adult liver fluke *Fasciola hepatica* was found. Molecular techniques were used in Laboratory Departamento de Parasitología, Facultad de Farmacia, Universidad de Valencia, Spain to verify that the liver fluke was *Fasciola hepatica*.

The results were subjected to statistical analysis using χ^2 -test. χ^2 -test was used to determine the significance of differences in mean prevalence of *Fasciola hepatica* in liver of bison in researched years. The samples met the criteria at $P \leq 0.05$.

Results

The analyzed ITS-1 and ITS-2 sequences of nuclear DNA have a length of 433bp and an AT content of 47.8 % and a length of 365 bp and an AT content of 51.2 % respectively. The prevalence of invasion of the liver fluke *F. hepatica* in the ducts bile of liver of bison in the 2001 – 2006 is presented in Table 1, Figures 1and 2. In winter from December 2001 to March 2002 out of 35 bison specimens involved in the study, 6 (17.1 %) individuals including as follow: 2 (5.7 %) adult and 4 (11.4 %) calves had liver flukes, which amounted 17.1 % of tested bison population. From December 2002 to March 2003 from among 40 bison, the liver fluke was detected in 15 (37.5 %) individuals including as follow: 12 (30 %) adults, 2 (5 %) juveniles and 1 (2.5 %) calf. Prevalence of infection was 37.5 % of population.

From October 2003 to March 2004 out of 35 specimens examined, 11 (31.4 %) individuals including as follow: 8 (22.8 %) adults and 3 (8.5 %) juveniles were infected by parasites, which amounted 31.4 % of tested animals.

Of the 34 bison examined from December 2004 to April 2005, only 9 (26.4 %) adult animals were infected by liver fluke. Prevalence of infection was 26.4 % of tested population.

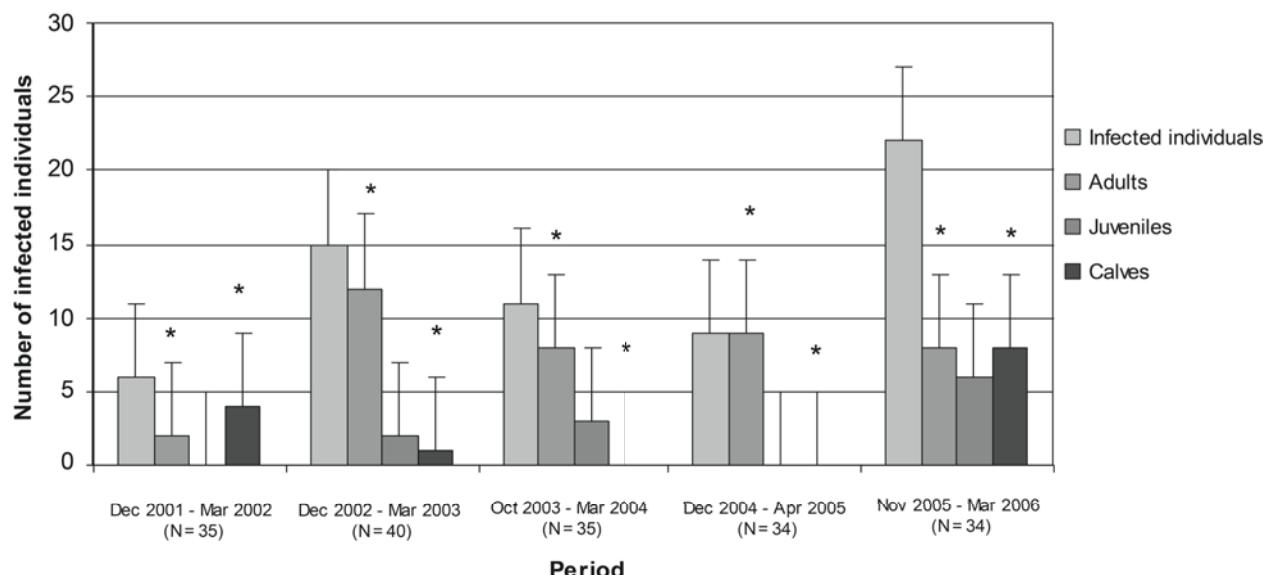


Fig. 1. The age effect of the infection of *Fasciola hepatica* in Bison bonasus in Białowieża National Park, 2001 – 2006.
Significance of differences in statistical analysis * $P \leq 0.05$.

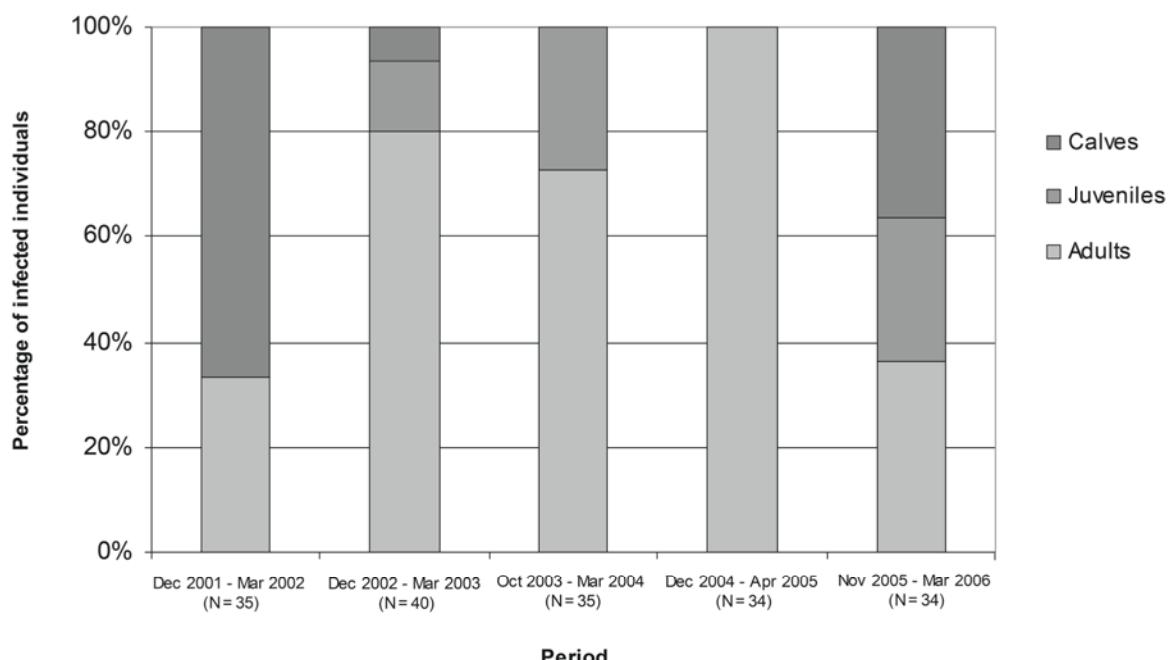


Fig. 2. Prevalence of Bison bonasus in each age groups infected by *Fasciola hepatica* in Białowieża National Park, 2001 – 2006

From November 2005 to March 2006 anatomized 34 of bison. Liver fluke was found in 22 (64.7 %) individuals including as follow: in 8 (23.5 %) adult and 6 (17.6 %) juveniles, 8 (23.5 %) calves.

In the 2001 – 2006, among 178 bison the liver fluke *F. hepatica* was found in ducts bile of liver of 63 (35.3 %) individuals including as follow: in 39 (21.9 %) adults, 11 (6.1 %) juveniles and 13 (7.3 %) calves. The main result of our study was that prevalence of infection of liver fluke *F. hepatica*, among European bison *Bison bonasus* in Białowieża National Park between 2001 – 2006 oscillated about 35.3 %. The most extensive invasion of fascioliasis was observed in adult animals (21.9 %). The most results were statistically significant ($P \leq 0.05$). The prevalence of *F. hepatica* invasions was significant in 2001 – 2006 in age groups of bison as follow: adults ($P = 0.0001$) and calves ($P = 0.0156$).

Discussion

Fascioliasis is a parasitic disease caused by the trematodes *Fasciola hepatica* and *Fasciola gigantica* that affects both animals and humans. *Fasciola hepatica* has a worldwide distribution with a high prevalence in livestock in different endemic areas in the world. On the other hand, *F. gigantica* is more limited to tropical areas, namely Africa, the Middle East, Eastern Europe and Eastern and Southern Asia. In Europe, fascioliasis is considered an endemic disease in farm animals, even though its distribution may be extended, as well as its infective capability, due to its ability to affect wild herbivores and rodents with different degrees of intensity (Mas-Coma *et al.*, 2009). The European bison (*Bison bonasus*) is the most of Asia but presently it is limited to reserves and parks of Poland and Belarus

(Krasinska & Krasinski, 2004).

The material for macroscopic and molecular analysis of liver fluke specimens was obtained from bile ducts of liver of bison. It should have been noticed that bison in the anatomized population were wild living under pressure of natural selection. Natural selection affects old individuals much more than young ones due to characteristic decrease of body weight and receptive in infections. A genotypic analysis of the internal transcribed spacers ITS-1 and ITS-2 of the nuclear DNA of adult liver flukes obtained by necropsy of wild bison from Białowieża National Park, Poland was performed. The analyzed ITS-1 and ITS-2 sequences have a length of 433bp and an AT content of 47.8 % and a length of 365 bp and an AT content of 51.2 % respectively. The comparison of the ITS-1 sequences to other sequences published for *F. hepatica* demonstrate that the analyzed sequences are identical to those of the genotype for *F. hepatica*. The comparison of the ITS-2 sequences published for both *F. hepatica* and *F. gigantica*, from other hosts and other countries, also demonstrate complete homology with European original genotype for *F. hepatica* that differentiate it from the genotype of *F. gigantica* (Artigas *et al.*, 2004; Semyenova *et al.*, 2005; Mas-Coma *et al.*, 2009).

Within the studied area of Białowieża National Park the prevalence liver fluke-induced infection has become lower, compared with the 90s of the previous century (Dróżdż *et al.*, 2002). This is probably the result of weather – dry and hot summers. Four free living European bison shot in 1997 within Lutowiska Forestry District in Bieszczady Mountains, Poland were infected with 9 species of nematode. Among all necropsies animals only in the excrement of one of them eggs of *Fasciola hepatica* were found (Dróżdż *et al.*, 2000). Fascioliasis in Poland occurs in different inten-

sity, which is firstly dependent of region and season when the research was done. Earlier observation of cattle in Warmińsko-Mazurskie Voivodeship, Poland has also revealed a decline in the prevalence of infection by liver fluke in 1980 – 1991. *Fasciola hepatica* was found in 28.2 % of the population of animals (Uradziński & Radkowski, 1992). Recently visible increase of infection by *Fasciola hepatica* in this area has been showed (Michalski & Romaniuk, 2000).

The investigations on *Fasciola hepatica* presence in cattle of Lubelskie Voivodeship, Poland demonstrated different results. Within studied area prevalence of infection of liver fluke in cattle underwent in lately of years considerable growth in comparison to 90 years past of century. In the next period prevalence of infection of *Fasciola hepatica* in that field was lower – between 4.1 – 24.3 %.

Liver fluke occurs in bison very often and in average 44 % of researched animals are infected (Deryło *et al.*, 2001). Strong invasion of this parasite gives clear clinical manifestation in bison like thinning down, dysentery and poor growing. Parasite is common between domestic ruminants. In North America, *Fasciola hepatica* is commonly found in domestic ruminants also but is uncommon in wild ruminants (Pybus, 2001). In bison *Fasciola hepatica* has been reported from one American bison in Montana, USA (Locker, 1953) and one American bison in Wyoming, USA (Bergstrom, 1967). The American bison is a native ruminant of North America and is a host of numerous parasites that also infect a variety of other wild and domestic ruminants. The experimental study was conducted by Foreyt and Drew (2010) to evaluate the susceptibility of American bison to liver flukes *Fasciola hepatica* and *Fascioloides magna*. Based on these results, bison are highly susceptible hosts for *Fasciola hepatica* because all inoculated bison became infected. Therefore evaluation for *Fasciola hepatica* infection should be accomplished in enzootic areas where bison are pastured in the vicinity of infected cattle, sheep or goats. It is suggested that reinvasion between bison, cattle, sheep and goats on common grass-lands can happen (Kiziewicz *et. al.*, 2004; Krasińska & Krasiński, 2004; Foreyt & Drew, 2010). The results referring to infection of bison within the Białowieża National Park, Poland of *Fasciola hepatica* are very interesting. The epidemiological importance of these finding is evident, demonstrating the fascioliasis expansion in restricted or protected areas, as National Parks such as Białowieża National Park, habitat of this wild host, and where no domestic animals are present. It is suggested to continue studies on liver fluke *Fasciola hepatica* not only on domestic ruminants but also on wild animals.

Conclusions

The obtained results referring to infection of bison within the Białowieża National Park, Poland of *F. hepatica* are very interesting. The epidemiological importance of these finding is evident, demonstrating the fascioliasis expansion in restricted or protected areas, as National Parks such as

Białowieża National Park, habitat of this wild host, and where no domestic animals are present.

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