

# Importance of the Danube River in spreading the infection of red deer with *Fascioloides magna* in eastern Croatia

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**Keywords:** Danube, *Fascioloides magna*, eastern Croatia, red deer

## 1 Introduction

Large American liver fluke (*Fascioloides magna*) is a trematode which lives in the liver parenchyma of various wild and domestic ruminants, causing a disease - fascioloidosis. This parasite, native to North America (Mulvey 1991), has been introduced to Europe with wapiti deer (*Cervus elaphus canadensis*) through Italy in 1865. It was sporadically found in Cuba (Lorenzo et al. 1989), South Africa and Australia. From Italy it spread in the 1930s to Germany and Poland, and further to the Czech Republic (in 1960), Austria, Slovakia and Hungary (Erhardova-Kotrla 1971; Rajskey et al. 1994; Majoros & Sztojkov 1994; Ursprung et al. 2006). This specific distribution route mainly follows the Danube River course. It was suggested that *Fascioloides magna* was introduced from Slovakia to Hungary during the seasonal migration of red deer. The occurrence is also confined to the Danube inundation area due to presence of an intermediate host, freshwater snail *Lymnaea truncatula* (Špakulová et al. 1997).

Based on the migration routes of red deer, it is anticipated that fluke will reach the Croatian territory. It was recorded for the first time in January 2000, during liver examination of shot red deer from the Danube region (Marinculić et al. 2002). The aim of this study was to indicate the importance of the Danube River in distribution of *Fascioloides magna* in Croatia, and to foresee its further expansion.

Although many species are susceptible to infection, the common definitive hosts of the fluke in North America are wapiti (*Cervus elaphus canadensis*), white-tailed deer (*Odocoileus virginianus*) and caribou (*Rangifer tarandus*), while in Europe it occurs commonly in red deer (*Cervus elaphus*), fallow deer (*Dama dama*) and roe deer (*Capreolus capreolus*). Among domestic animals, cattle are parasitized commonly, but usually are aberrant dead-end hosts, while infection is usually fatal in sheep and goat (Foreyt 1990). First case of *Fascioloides magna* infection in horse was reported by McClanahan et al. (2005) from a horse grazing in a marshy area of Central Minnesota (USA). Infection of the final host occurs via ingestion of metacercariae from herbage. Juvenile flukes emerge from the metacercariae in the stomach and intestine and migrate through the abdominal cavity into the liver. Mature flukes are enclosed in fibrous capsules within the liver parenchyma. The capsule contains a great mass of eggs and is connected to bile-ducts. The eggs are passed together with bile into the bile collecting system, enter the small intestine, and leave the definitive host along with the faeces. Some of the clinical signs are lethargy, weight loss and decreased quality of antlers in cervids.

## 2 Materials and methods

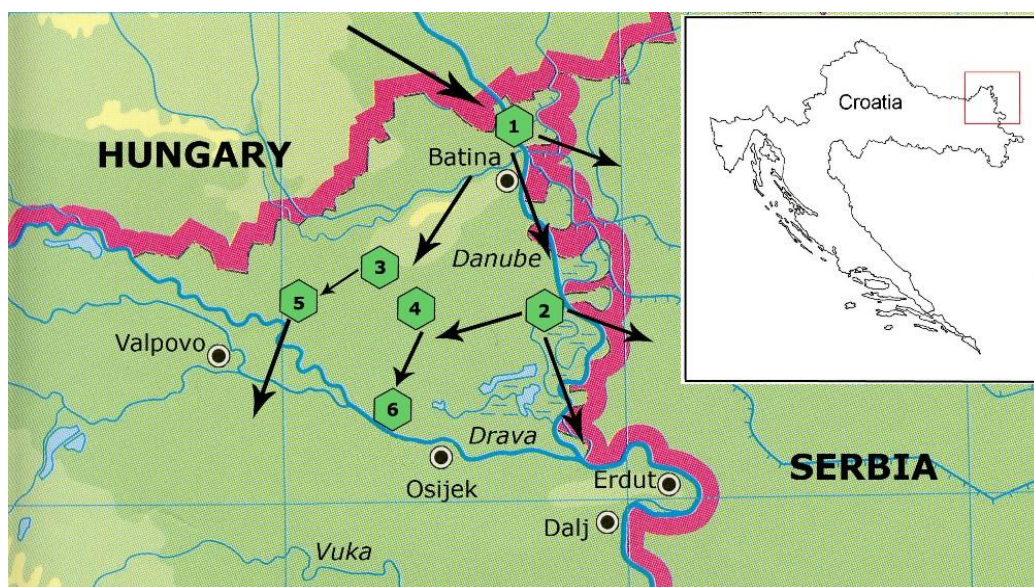
Faecal samples of red deer (*Cervus elaphus* L.) were collected from 2001 to 2004 in six hunting grounds in Baranja region in eastern Croatia (Figure 1). Along the Danube course are situated: "Šarkanj-Vrblje" (rkm 1433-1426) and "Podunavlje-Podravljje" (rkm 1425-1382), in which *Fascioloides magna* was recorded for the first time

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in Croatia. Two hunting grounds: “Haljevo” and “Koha-Kozarac” are situated in the middle part of Baranja, on higher level outside the reach of floods. The remaining two: “Podravlje” and “Munjoroš” are situated along the left bank of the Drava River, upstream of the City of Osijek.

Sedimentation technique and modified McMaster method (Thienpont et al. 1979) were used for the diagnosis and estimating *Fascioloides magna* eggs in the faecal samples. Eggs were detected and counted in Petri dishes using a dissecting microscope (magnification 100-200 x). Arithmetical mean of eggs per gram of faeces, and prevalence as relative amount of positive samples were calculated for each locality. The statistical analysis was carried out by the computer program STATISTICA (StatSoft 2005).



**Figure 1.** Map of the study area. Numbers indicate the location of the investigated hunting grounds: 1-“Šarkanj-Vrblje”; 2-“Podunavlje-Podravlje”; 3-“Haljevo”; 4-“Koha-Kozarac”; 5-“Podravlje”; 6-“Munjoroš”. Arrows indicate routes of *Fascioloides magna* spreading.

### 3 Results and discussion

First evidence of fascioloidosis in Croatia arose in winter 1999, when an unusual weaker constitution was observed in individuals of the red deer (*Cervus elaphus* L.) free ranging population in Baranja region (Marinculić et al. 2002). Liver from individuals shot in January 2000 was examined at Faculty of Veterinary Medicine in Zagreb, and 22 adult parasite *Fascioloides magna* were found. In order to estimate the degree of invasion in red deer, a systematic monitoring was carried out in the period 2001-2004. The chosen priority area was Croatian Danube region, in particular hunting grounds in the flooded area.

High prevalence of fascioloidosis in red deer population was recorded in hunting grounds located along the Danube course (Table 1). In „Podunavlje-Podravlje“, the prevalence was 44-52% (mean 48%), and in „Šarkanj-Vrblje“ 35-60% (mean 46%). In comparison, Špakulova et al. (1996) reported a prevalence of 70% in Slovakia during winter season 1995-1996. Gradual reduction of prevalence from 2001 to 2004 is a consequence of intensive treatment of red deer with mixture of triclabendazole and standard deer salt brick components, at a dose of 60 mg/kg body weight per deer, twice in seven days.

Fascioloidosis was not detected in hunting grounds along the Drava River during monitoring in 2001-2002, but it was confirmed in 2003 and 2004 with low prevalence of 13% in „Podravlje“ and 7% in „Munjoroš“. This proves that in Baranja well-established migration routes of red deer lead from the Danube floodplains to the Drava River inundation, and across the Drava to Slavonia.

No *Fascioloides magna* eggs were found in faeces of red deer in "Haljevo" and "Koha-Kozarac" with the exception of two samples from „Koha-Kozarac" in 2004, with a prevalence of 7%.

This part of Baranja is located at higher terrain which is outside of the reach of floods and not hydrologically connected to the Danube floodplain. These hunting grounds provide an intermediate stop on the red deer migration route, mainly during the winter period. Thus, the only possible way of spreading fascioloidosis is by seasonal migration of red deer.

**Table 1.** General data and prevalence of fascioloidosis in red deer population in selected hunting grounds of eastern Croatia

Hunting ground	Total surface (ha)	Altitude range (m a.s.l.)	Year	Number of samples		Mean number of eggs per gram $\pm$ SD	Prevalence of fascioloidosis	
				Collected	Positive		(%)	Mean (%)
XIV/11 Šarkanj-Vrblje	1,337	85-86	2001	15	7	47.43 $\pm$ 27.39	47	46
			2002	60	36	46.08 $\pm$ 33.93	60	
			2003	60	26	20.88 $\pm$ 11.68	43	
			2004	60	21	10.87 $\pm$ 6.03	35	
XIV/9 Podunavlje-Podravlje	25,333	82-86	2001	247	129	51.01 $\pm$ 51.84	52	48
			2002	300	151	32.97 $\pm$ 23.64	50	
			2003	300	139	23.91 $\pm$ 14.11	46	
			2004	300	133	25.69 $\pm$ 13.35	44	
XIV/5 Koha-Kozarac	1,293	89-90	2001	4	0	0	0	7
			2002	30	0	0	0	
			2003	30	0	0	0	
			2004	30	2	2 $\pm$ 0	7	
XIV/3 Haljevo	1,681	90-101	2001	0	0	0	0	0
			2002	30	0	0	0	
			2003	30	0	0	0	
			2004	30	0	0	0	
XIV/10A Podravlje	8,410	86-90	2001	0	0	0	0	13
			2002	30	0	0	0	
			2003	30	4	5.50 $\pm$ 2.88	13	
			2004	30	4	3.25 $\pm$ 1.50	13	
XIV/10 Munjoroš	2,400	85-88	2001	5	0	0	0	3
			2002	45	0	0	0	
			2003	45	1	1 $\pm$ 0	2	
			2004	45	2	2 $\pm$ 0	4	

Relatively high prevalence of fascioloidosis in hunting grounds located along the Danube River can be explained by their terrestrial and hydrological connection. These hunting grounds have similar ecological and habitat characteristics, with optimal conditions for the same red deer population (Florijančić & Ozimec 2004). The frequency of disease depends on population density, which is important for the estimation of epizootiological situation. In „Podunavlje-Podravlje“, the largest hunting ground by its surface, density is 8 individuals per 100 ha of hunting surface, and 2 individuals per 100 ha in „Šarkanj-Vrblje“, reflecting prevalence differences.

The Danube hydrologically connects the upstream Hungarian hunting grounds and Croatian hunting grounds in which fascioloidosis occurred. It can be confirmed that developmental stages of *Fascioloides magna* were introduced in Croatia by migration of red deer, and by intermediate host – freshwater snails deposited with floods during the high water-level episodes.

This corresponds to the description of introduction from Slovakia into Hungary (Mayoros & Sztojckov 1994). Aquatic and wetland habitats favour the growth of the freshwater snail *Lymnaea truncatula*; its high presence is noticeable in the flooded area of the hunting grounds.

The risk of spreading fascioloidosis in red deer populations in eastern Croatia is still present, when considering that eggs of the parasite may lie dormant in the environment, and may be carried away at distant sites by flooding. The hunting grounds in the middle part of Baranja and along the Drava River can be treated as endangered areas regarding fascioloidosis. Metacercariae can survive in snails for a long time, and can survive the cold winter period, keeping vitality up to one year (Griffiths & Christensen 1972). Although the fertility of the liverfluke decreases with maturity, deer which are invaded only once can excrete eggs until the end of life (Foreyt et al. 1977).

## 4 Conclusions

Distribution of American giant liver fluke (*Fascioloides magna*) in red deer, with rather strong invasion, is limited on the Danube flooded area in eastern part of Croatia. The ways of spreading are by seasonal migration of infested red deer through the area, and by transport of an intermediate host, the freshwater snail *Lymnaea truncatula*, by high flow. Low prevalence of fascioloidosis was found in flooded areas along the Drava River, while the lowest was at higher terrain outside of the floodplain. Considering the migration routes of red deer and epizootic indicators, the spreading of this parasitic disease is expected across the Danube to the left riverside in Serbia, as well as in hunting grounds across the Drava River in Croatia.

Regular parasitological monitoring (sampling, analyses of faeces and liver) are needed in the epizootic areas of the Danube region, but also in the endangered areas where a disease was confirmed. Proper application of zoohygienic measures in hunting grounds, combined with antiparasitic treatment of free ranging red deer population and captive deer is important to restrain the further spread of fascioloidosis.

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