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Treatment of fascioloidosis (*Fascioloides magna*, Bassi, 1875) in free ranging and captive red deer (*Cervus elaphus* L.) at eastern Croatia

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ABSTRACT

After the large American liver fluke (*Fascioloides magna*) was detected in Croatia for the first time in 2000, treatment of fascioloidosis was conducted in the free-ranging population over a period of three years (from 2001 to 2003) and in captive red deer during the winter seson of 2002. The free-ranging red deer population was treated with a mixture of triclabendazole and standard deer salt brick components at a dose of 60 mg/kg body weight per deer, twice in seven days. Captive deer were treated individually by intra-ruminal application of triclabendazole using a needle or oesophageal tube. Coprological control was made 30 days after administration of antiparasitic preparation. Positive results (findings of *F. magna* eggs in faeces) in the free ranging deer population varied from 20% to 80% respectively. In captive deer we recorded almost ideal efficiency of triclabendazole (after treatment 95.5% of all faecal samples was free of *F. magna* eggs).

Key words: red deer, large American liver fluke, *Fascioloides magna*, antiparasitic treatment, triclabendazole

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Introduction

The large American liver fluke (*Fascioloides magna*) is a well-known parasite (STILES and HASSALL, 1894) of primarily North American wild and domestic ruminants (FRANCIS, 1891). At the end of the nineteenth century autochthonous deer species from northern part of American continent were imported into Europe (HALL, 1912). These species, primarily white-tailed deer (*Odocoileus virginianus*) and wapiti (*Cervus elaphus canadiensis*) spread the Large American liver fluke through Europe and infected autochthonous red deer (*Cervus elaphus*).

Fascioloides magna was detected for the first time in Italy (BASSI, 1875) and after that in other European countries – Germany (SALOMON, 1932), Poland (SLUSARSKI, 1955), the former Czechoslovakia (ERHARDOVA-KOTRLA, 1971), Austria (PFEIFFER, 1983), Slovakia (RAJSKY et al., 1994) and Hungary (SZTOJKOV et al., 1995). In Croatia the first case of fascioloidosis was described by MARINCULIĆ et al. (2002), who reported that infected red deer originated from the Čiprašat area (45° 46' N; 18° 52' E) in the eastern part of country - the Baranja region. Parasites entered Croatia following the Danube downstream, a river which flows through most countries with persisting problems of *F. magna* occurrence.

Horizontal migrations of European red deer are well known (ERHARDOVA, 1961), and deer usually migrate along riversides from one habitat to another. MARINCULIĆ et al. (2002) presumed that *F. magna* was carried into eastern Croatia by the infected red deer population from neighboring Hungary. Another possibility is migration of different stages of parasite life cycle (eggs, intermediate host or metacercaria) flooded by water from the Danube. Local conditions of wetland habitats with shallow, medium temperate and slightly alkaline water are very suitable for the *F. magna* and its intermediate hosts (snails *Lymnea* spp.). These same conditions of such areas are also very attractive for cervids, so MULVEY et al. (1991) found that prevalence of *F. magna* in the free-ranging red deer population showed a tendency to be higher in swamp habitats than in dry uplands.

In the Baranja region, which is a typical lowland habitat, the free-ranging population of red deer is relatively abundant (around 800 specimens) and the risk of parasite dissemination through the region is reasonably high. That was the main reason why scientists and veterinary practitioners gathered together to prevent potential transmission of *F. magna* to other game species and domestic livestock. Immediately after detection of the parasite in 2000 an initial plan for monitoring and control of large American liver fluke was prepared and realized in practice over a three year period (2001 - 2003). The obtained results and field experience are presented in this paper.

Materials and methods

In the lowland area investigated, systematic collecting of red deer faecal samples was organized at 17 different positions in the Baranja region. During the winters of 2000/2001, 01/02 and 02/03 in habitats along the Danube River a total of 755 fecal samples were collected from the deer feeding spots (total deer population in researched area was estimated at 1500 individuals). All fecal samples were examined by the standard fecal analysis procedure at the Department of Parasitology and Parasitic Diseases, of the Faculty of Veterinary Medicine, University of Zagreb. The sedimentation method (VARADY, 1993) was used and the EPG (eggs per gram of faeces) of large American liver fluke eggs was calculated for every sample.

Due to the fact that adult mature flukes may release up to four thousand thick-walled operculate eggs per day (SWALES, 1935), preventive therapy of fascioloidosis in the endangered Baranja region had to been done as soon as possible. Prophylactic treatment of infected deer could be carried out in two ways - group therapy, as well as an individual therapy.

For the free-ranging red deer population we applied group therapy – per os application of anthelminthic agents incorporated in suitable bait. While most researchers used only corn as bait (QURESHI et al., 1989; PYBUS et al., 1991; QURESHI et al., 1994) in our experimental treatment we used a mixture of salt and flukecides. One kilogram of salt mixture was composed of: 2.5% plaster ($CaSO_4$); 60% salt (NaCl); 10% chalk ($CaCO_3$); 17.8% lime ($Ca(OH)_2$); 9.7% 'Benal® - Boehringer Ingelheim Vetmedica GmbH' (inert powder); 720 ml ''Fasinex® - Novartis AHC Inc.'' 10% (100 mg/ml triclabendazole) suspension. After the mixing of all components, the preparation was finished by drying for 24 hours – at a maximum temperature of 40 °C. Finally one kg of this mixture contained 72 g triclabendazole, with 6 g representing a single dose for red deer with average weight of 100 kg (a quantity of 60 mg/kg body weight per deer was calculated as an efficient dose). This means that one kilogram of salt mixture was sufficient to feed approximately 10 to 12 adult deer. During the winter months – January and February (three yrs in total), the mixture was exposed to the free-ranging deer on 17 feeding tables, with some corn grain on top to increase attractiveness (JANICKI et al., 2005). Consumption was mostly finished in 48 hours from exposure. After seven days second treatment/exposure was made and coprological control was done 30 days after the last treatment.

Individual therapy was carried out on 17 animals (1 stag and 16 hinds) during February 2002, in the fenced area "Zlatna Greda". All captive deer specimens were weighed preciously (tension helical balance ''Titan'') and body mass was determined. According to the body weight (b.w.), the effective dose of triclabendazole at 60 mg/kg b.w. was defined repeatedly for each specimen. To those animals the drug was delivered directly by

intraruminal administration, in two different methods of application – by long needle and by oesophageal tube. Both methods of application required chemical immobilization of animal and we used a tiletamine-zolazepam and xylazine hydrochloride mixture (JANICKI et al., 2006). After the treatment, all deer were kept in a fenced enclosure for 30 days and then re-examined for the presence of *F. magna* eggs by faeces examination control.

Results and discussion

During the three years of the monitoring period (2001 – 2003) coprological analysis of the free-ranging deer population showed a remarkable percentage of positive faecal samples (Table 1).

Table 1. Percentage of positive faecal samples of free-ranging deer population during three years period (2001 - 2003) of coprological monitoring in Baranja region

Location	Year 2001	Year 2002	Year 2003
No. Name	Positive Samples	Positive Samples	Positive Samples
1. Aleksina	33%	31%	29%
2. Hranilište	43%	40%	37%
3. Dvorac I.	77%	67%	58%
4. Dvorac Ii.	80%	57%	44%
5. Zlatna Greda	30%	30%	27%
6. Oldobreda	53%	50%	47%
7. Čakanjska	61%	60%	55%
8. Ludoš I.	57%	53%	50%
9. Ludoš II.	41%	40%	38%
10. Bat Siget	57%	55%	51%
11. Vratić	35%	30%	29%
12. Ludoš II.	50%	48%	46%
13. Siget	26%	22%	20%
14. Bat I.	39%	37%	31%
15. Bat II.	58%	57%	52%
16. Bat III.	56%	54%	50%
17. Bat IV.	59%	58%	54%

On most collecting positions the percentage of positive samples was similar, while at position three (Dvorac I – Gabrijela) and four (Dvorac II - Livada) we recorded a significant decline in the number of positive faecal samples over three years. The distribution of positive faecal samples presented in Fig. 1 showed a slightly decreasing tendency at all collecting spots, with a standard decrease in EPG (eggs per gram of faeces).

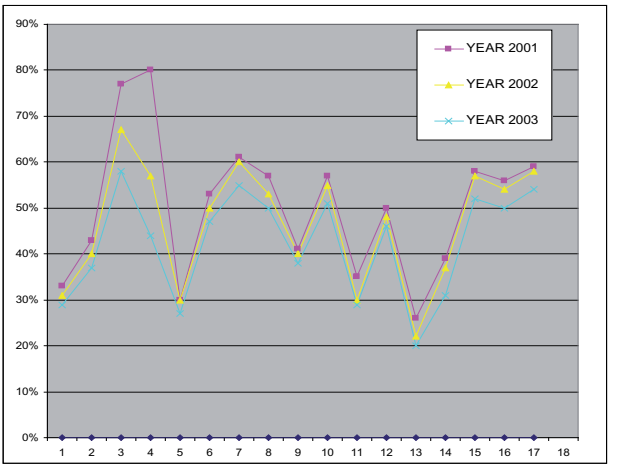

Fig. 1. Distribution of positive faecal samples on 17 collecting spots during the monitoring period (2001 - 2003) in Baranja region

Before group therapy in 2001 we recorded 143 positive samples (54.1%) from the total number of 264 examined faecal samples. In all positive samples 88 of them (61.5%) had EPG > 5, and in 55 samples (38.5%) we found EPG < 5. After the group therapy in 2003 we recorded 98 positive samples (41.0%) from total number of 239 faecal samples examined. In all positive samples 24 of them (24.5%) had EPG > 5, and in 74 samples (75.5%) we found EPG < 5. The same effect of triclabendazole therapy is reported by other authors (BALBO et al., 1989; QURESHI et al., 1989; PYBUS et al., 1991; QURESHI et al., 1994). Moreover, BALBO et al. (1987) reported a temporary reduction of EPG in red deer after treatment with rafoxanide (15 mg/kg) and 45% reduction of EPG after treatment with triclabendazole. All group therapies have to solve the same problem – how to reach the proper dosage of antiparasitic preparation without occurrence of over-dosing in the dominant deer specimens. Another problem is the fact that *F. magna* infections are difficult to treat (PYBUS, 2001) because in the hosts' organism flukes are not directly within the bile ducts, they are encapsulated in the

liver parenchyma and sufficient amounts of anthelminthics hardly get into the capsules. Triclabendazole was proved as the most effective drug, with equal efficiency on the mature and juvenile stages of large American fluke, but efficacy and dosage also depend of the method of drug administration. The method of offering triclabendazole in feed (per/oral application) as a group therapy, could be useful only for prophylactic purposes.

In fenced area collective droppings were analyzed before and after the treatment with flukecides (Table 2).

Table 2. Results of group coprological monitoring before and after therapy

	Examined Samples	Positive Samp.	Negative Samp.
Before Therapy	22 (17 ♀ + 5 ♂)	15 ♀ + 3 ♂ (81.8%)	2 ♀ + 2 ♂ (18.2%)
After Therapy	22 (17 ♀ + 5 ♂)	1 ♀ + 0 ♂ (4.5%)	16 ♀ + 5 ♂ (95.5%)

Table 3. Values of EPG (eggs per gram) in hinds faeces before and after the therapy

Number of *F. magna* eggs in faeces (EPG)		
No.	Before treatment	After treatment
1.	30	0
2.	9	0
3.	5	0
4.	250	0
5.	3	0
6.	8	0
7.	0	0
8.	10	0
9.	160	0
10.	120	0
11.	7	0
12.	0	0
13.	6	0
14.	4	0
15.	2	0
16.	30	0
17.	300	3

Before the therapy *F. magna* eggs were detected in 14 samples (87.5%) from hinds. After therapy only one sample of hinds contained *F. magna* eggs (6.25%) in very low concentration (EPG < 2). Individual coprological tests showed that six hinds have numerous eggs of *F. magna* (EPG > 10) in faecal samples (Table 3).

These hinds were treated one by one and results of individual therapy show that Triclabendazole at dosage of 60 mg/kg b.w. is very effective in European red deer against reproductive active *F. magna* specimens (Table 3). The efficacy of Triclabendazole in individually treated hinds was high (94.75% samples were free of *F. magna* eggs) and reduction of EPG was significant.

Conclusions

Health management of autochthonic red deer in Croatia has to implement ''System of integrated control for large American fluke''. This system includes five main points:

1. 'Up-to-date' coprological monitoring and permanent, complete health monitoring
2. Group preventive therapy
3. Individual therapy
4. Quarantine measures (considering prepatent period of min. 30 days)
5. Interrupting the parasite's life cycle

Due to numerous limitations which affect the natural biotope with interventions on the intermediate hosts (snails *Lymnea* spp.) the last point of the protocol had to be skipped, so first four points had to achieve successful control of fascioloidosis. Strategically the most important task is to prompt coprological monitoring in lowland wet habitats where F. magna persists. In the Croatia group preventive therapy achieved success in restricting the F. magna infection exclusively to red deer, despite increased contacts with other wild and domestic ruminants (fallow deer, cattle, and sheep) and the population explosion of wild boar in the Baranja region. In other endangered countries F. magna has spread to other domestic (HALL, 1912; PRICE, 1953; CAMPBELL and TODD, 1954; FOREYT and TODD, 1976; STROMBERG et al., 1985) and wild ruminants (KOTRLA and KOTRLY, 1977; KOTRLY and KOTRLA, 1980).

While treatment of the free-ranging deer population has to be resumed as preventive therapy, individual treatment is the only reliable method for suppressing *F. magna* infection (JANICKI et al., 2005). Of course, this therapy includes handling and chemical immobilisation of treated animals, so it cannot be used in every day practice. Due to these limiting factors of individual treatment, such therapy has to be restricted to animals subjected to transport or translocation.

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SAŽETAK

Nakon što je pojava velikoga američkoga metilja (*Fascioloides magna*) prvi puta potvrđena na tlu Republike Hrvatske tijekom 2000. godine, poduzete su prve mjere za suzbijanje fascioloidoze. Skupno liječenje slobodno živućih jelena antitrematodnim sredstvima provođeno je tijekom tri godine (2001. – 2003.), dok je pojedinačno liječenje u ograđenom prostoru izvedeno tijekom zimskih mjeseci 2002. godine. Jelenima koji slobodno žive u prirodnim uvjetima ponuđena je tijekom zime kombinacija triklabendazola i mineralne soli, u dozi od 60 mg djelatne tvari po kilogramu tjelesne mase jelena. Peletirana hrana izlagana je na hranilištima

jelenske divljači dvokratno, s razmakom od sedam dana. Jeleni u ograđenom prostoru liječeni su pojedinačno na način da im je triklabendazol apliciran izravno u burag pomoću sonde ili specifične dugačke igle. Koprološka pretraga napravljena je trideset dana nakon posljednjega liječenja jelenske divljači. Pozitivne rezultate pretrage, odnosno pronalazak jajašaca velikoga američkoga metilja u izmetu slobodno živućih jelena utvrdili smo u relativno visokom postotku (20 do 80 %). U jelena s ograničenom slobodom kretanja (ograđeni uzgoj) zabilježili smo vrlo visoku učinkovitost triklabendazola, tako da je nakon terapije 95,5 % pregledanih uzoraka jelenskog izmeta bilo negativno na prisutnost jajašaca velikoga američkoga metilja.

Ključne riječi: jelen obični, veliki američki metilj, *Fascioloides magna*, antiparazitska terapija, triklabendazol