

Evaluation of factors affecting the efficacy of treatment against *Fascioloides magna* in wild red deer population

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Red deer,
Fascioloides magna,
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Disease control program,
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Summary

Deer fascioloidosis is a serious and potentially fatal disease caused by the non-native trematode *Fascioloides magna*. Infections of red deer with *F. magna* in Croatia have been reported for the first time in 2000 in the Baranja region. Subsequently, the disease spread throughout the Eastern parts of the country, involving all 3 deer species (red, roe, and fallow) and mouflons. Within the disease control programme (DCP), livers from all shot deer were thoroughly analysed and all detected trematodes and gross lesions were counted and categorized. Prevalence of positive animals, in this study for Spačva region, in the period ranging from 2007 to 2012 was 36.42% (46.39% when fawns are not considered). Epidemiological analysis was applied to evaluate risk factors and disease patterns at the population level with the aim to understand factors with negative influence on therapeutic effect. Each demographic variable was tested at the seasonal, individual and location level. Model for pathological lesions suggested that the likelihood of lesions was dependent on age ($p = 0.003$). We did not find any locality or sex related significant differences. Finally, environmental characteristics and migratory patterns were analysed using Geographic Information System (GIS) and showed that Spačva region represents an epidemiological unit for red deer fascioloidosis.

Valutazione dei fattori che influiscono sull'efficacia del trattamento per la *Fascioloides magna* in popolazioni di cervi rossi selvatici

Parole chiave

Cervo rosso,
Croatia orientale,
Epidemiologia,
Fascioloides magna,
Programma di controllo
della malattia.

Riassunto

La fascioloidosi dei cervi è una malattia grave e potenzialmente fatale causata dal trematode *Fascioloides magna*. In Croazia i cervi rossi sono stati infestati per la prima volta nella regione di Baranja nel 2000; la malattia si è diffusa successivamente interessando la parte orientale del paese, coinvolgendo il cervo rosso, il capriolo, il daino e i mufloni. Nell'ambito del programma di controllo delle malattie (DCP), sono stati accuratamente analizzati i fegati di tutti i cervi e tutti i trematodi individuati così come le lesioni evidenti sono stati contati e classificati. In questo studio, effettuato tra il 2007 e il 2011 nella regione di Spačva, la prevalenza degli animali positivi è stata del 36,42% (46,39% se si escludono i cerbiatti). Per comprendere i fattori che influenzano negativamente i risultati terapeutici, è stata condotta un'analisi epidemiologica che ha valutato i fattori di rischio e la distribuzione della malattia nella popolazione, analizzando tutte le variabili demografiche a livello stagionale, individuale e geografico. Il modello sulle lesioni patologiche ha evidenziato che la probabilità di avere lesioni dipende dall'età ($p = 0,003$), mentre non si è riscontrata alcuna differenza significativa legata a posizione geografica o sesso. Infine, le caratteristiche ambientali e i movimenti migratori, analizzati utilizzando GIS, hanno dimostrato che la regione di Spačva può essere considerata come un'unità epidemiologica per quanto concerne la fascioloidosi dei cervi rossi.

Introduction

Fascioloidosis is a potentially fatal parasitic disease of various domestic and wild ruminants caused by the Digenean trematode *Fascioloides magna*. Originally a parasite of the North American deer species, *F. magna* was for the first time described in the 20th century in Europe. On the European continent, its presence has been recorded in the Czech Republic (Ullrich 1930, Erhardová-Kotrlá 1971, Kralová-Hromadová et al. 2011, Kasný et al. 2012), Austria (Pfeiffer 1983, Ursprung et al. 2006), Hungary (Majoros and Sztojokov 1994), Slovak Republic (Rajsky et al. 2002), Croatia (Marinculić et al. 2002), Germany (Salomon 1932), and Serbia (Trailović et al. 2008). Reports of *F. magna* presence in Europe followed the path of the Danube River, as it has been recently confirmed by a genetic study (Bazsaloviczsová et al. 2013). This pattern could be attributed to the fact that major European rivers offer favourable habitat conditions for sustaining large deer population, as well as conditions necessary for completion of the parasite life cycle. This is further supported by the fact that red deer exhibits high level of horizontal migration, especially during the rutting season, which is important for spreading of the parasite. Following its first description and initial devastating effect on the red deer population in Croatia [which was later even more emphasized in the case of the roe deer (*Capreolus capreolus*), fallow deer (*Dama dama*) and mouflon (*Ovis gmelini musimon*) populations], numerous efforts have been dedicated to monitoring and controlling this disease in Slavonia and Baranja region (Janicki et al. 2005, Janicki et al. 2012, Rajković-Janje et al. 2008).

However, it was expected that the outcome of the disease control program (DCP) and the efficacy of applied measures would be heavily influenced by various factors. The objective of this study was to determine the prevalence of infection with *F. magna* in free-ranging red deer population in Eastern Croatia during the study period and to evaluate demographic and environmental factors associated with the infection. The factors have been assessed using different approaches and focusing on sex and age of the animals; deer migration characteristics; proximity to state border and areas with deer populations without DCP; and environmental characteristics. All these factors were identified as those with potentially negative effects on the efficacy of DCP.

Material and methods

Study area, animals, and parasitological analysis

The study was conducted in the open state hunting ground XIV/11 "Spačva" located in Eastern

Croatia near the borders with Serbia, Bosnia, and Herzegovina. Spačva hunting ground spans over an area of 25,018 ha of predominantly oak (*Quercus robur*) forest, and is part of 51,000 ha large forest basin. It is lowland habitat with altitudes varying between 70 and 99 m a.s.l. Main game species are red deer, wild boar (*Sus scrofa*), and golden jackal (*Canis aureus*); while roe deer population significantly decreased since the outbreak of fascioloidosis. Sampling for this study strictly relied on the hunting regulations, and samples were collected following the hunting season. According to the instructions given in the management plan, the hunting bag includes deer of various ages in order to maintain proscribed age structure in the hunting ground, and approximately the same number of males and females as it is based on 1:1 sex ratio. In accordance with that, such sample can be characterized as a stratified random sample, particularly in the case of hinds and calves. Spring (basic) fund of red deer in Spačva hunting ground is approximately 550 animals, with calculated sex ratio 1:1. Sample size was determined with the aim to estimate the most likely prevalence of 50%, with 95% confidence, according to the standard approach for prevalence sample size calculation under random sampling (Dohoo et al. 2009). The sample size determined in this way was further adjusted using red deer spring fund as the finite population size. Calculated sample size of 447 animals, based on the spring fund was close to the sample determined by hunting management plan (n = 442).

Livers from each shot deer were collected, cut to approx. 2 cm thick slices and examined for gross lesions and fluke characteristics. Detected flukes were recorded as migrating stages, young and adult flukes. Gross lesions were, based on their characteristics, categorized as fluke migration paths, young cysts (thin walls, smooth inner surface), cysts (well-developed walls, traces of calcification on surface), degrading cysts (homogenized mass inside the cyst, flukes cannot be recovered) and scars. Each liver sample was accompanied with faecal sample, which was analysed using standard flotation method.

Epidemiological analysis

Two approaches were used to study the association between the infection status and explanatory variables. First, negative binomial regression models with location as a random effect were used to evaluate associations between the count variables – such as number of flukes in the liver sample and the number of eggs in the faeces as outcome variables – and demographic, environmental, and temporal variables. Second, logistic regression models with location as a random effect were used to assess the associations between presence of liver lesions and

eggs in the faeces and the same set of explanatory variables. Biologically plausible interactions were tested for statistical significance and contextual variables such as location-level prevalence were offered to the model when considered appropriate. Statistical analysis was conducted using Stata 10 (Stata, College Station, TX, USA). Number of observations was 442, divided in 26 groups (based on locations). The Mean number of observations per location was 17 (min 1, max 77, SD 22.3), while Median was 7 (IQR = 30). Results were considered significant when $P \leq 0.05$.

Geographic Information System analysis

Coordinates of localities at which red deer group reside (feeding stations) were taken using Garmin GPSmap 62s (Garmin Ltd., Olathe, Kansas, USA). Collected data were processed using ArcGIS 9.3 software.

Results

Parasitological findings

Within the period ranging between 2007 and 2012 we examined 442 shot red deer (on average 88 animals per year), whose carcasses were collected in the hunting ground in which DCP was applied. Within DCP, triclabendazole medicated baits were offered to animals during Spring (Janicki *et al.* 2005, Janicki *et al.* 2012). From 442 collected animals, 431 had a complete data set (including sex and age) and were included in the prevalence calculation.

Game managers indicated age categories on the basis of tooth characteristics (Wagenknecht 1979). Data on total number of examined deer and prevalence of positive animals per age category are given in Table I. Only livers without any fluke, cysts, scars or pigment (iron-porphyrin) traces were considered negative. Reinfected animals (previously negative or cured) were classified as positive. Total prevalence of infected animals was 36.43%. When calves were excluded from calculation prevalence rose to 46.39%. Cured animals (livers containing scars, iron-porphyrin pigment, degrading cysts or flukes, but without live flukes) participated in the total number with 14.15% and negative animals with 49.40%.

Epidemiological analysis

The number of flukes was associated with the age in a curvilinear fashion (Table II, Model 1, $P < 0.01$), after adjusting for the contextual effect of prevalence of pathological lesions. The expected values for the effect of age under condition of 15% and 50%

prevalence of pathological lesions in a specific location are depicted in Figure 1.

The number of fluke migratory stages was linearly associated with the age (Table II, Model 2, $P < 0.01$), after adjusting for the contextual effect of prevalence of pathological lesions and season. The expected values for the effect of age under condition of 15% and 50% prevalence of pathological lesions in a location in Autumn and Winter are depicted in Figure 2.

The probability of detecting typical pathological lesions was associated with age curvilinearly (Table II, Model 3, $P = 0.03$), after adjusting for the contextual effect of prevalence of pathological lesions in a specific location. However, the interaction between the linear effect of age and prevalence of pathological lesions was detected ($p < 0.01$). The expected probability of detecting pathological lesions in animals of different age is shown in Figure 3. The probability of having positive coprological test was linearly associated with age (Table II, Model 4, $P < 0.01$), after adjusting for the contextual effect of prevalence of pathological lesions in a specific location. The expected probability of having positive coprological tests in animals of different age is shown in Figure 4.

Regarding the association between animal's sex and risk of being positive, we did not find statistically significant difference ($\chi^2=2.01$) between males ($n = 253$, risk .454, CI 95%) and females ($n = 189$, risk .387, CI 95%). We also did not find correlation between location and severity of infections (Prob > chibar2 = 1.000).

Table I. Summary of total number of deer examined in this research (study period 2007-2012, Spačva area, East Croatia), including negative, positive and cured (livers containing scars, pigment traces, degrading cysts or flukes, without living flukes) animals and the prevalence of infected animals. Data are presented according to the age of the animals.

Age	Total	Negative	Infected	Cured	Prevalence (%)	95% CI
1	112	103	9	0	8.06	3.02-13.10
2	74	44	24	6	32.43	21.77-43.09
3	52	24	18	10	34.62	21.69-47.55
4	35	16	12	7	34.29	18.57-50.01
5	34	10	21	3	61.76	45.43-78.09
6	41	4	26	11	63.41	48.67-78.15
7	39	6	23	10	58.97	43.56-74.38
8	16	2	6	8	37.50	13.87-61.29
9	19	2	12	5	63.16	41.49-84.83
10	9	2	6	1	66.67	35.89-97.45
Total	431	213	157	61	36.43	31.99-40.87

Table II. Final multivariable random effect regression models for number of flukes (model 1), number of flukes migratory stages (model 2), likelihood of pathological lesions (model 3), and likelihood of positive coprological findings for fascioloidosis (model 4) in red deer in eastern Croatia (2007-2012).

Variable	Model 1 ^s				Model 2 ^s			
	Estimate	95% Confidence Interval		P	Estimate	95% Confidence Interval		P
Intercept	-4.61	-5.33	-3.90	<0.01	-2.82	-3.47	-2.18	<0.01
Age (years)	0.68	0.43	0.93	<0.01	0.18	0.12	0.24	<0.01
Age2 (years)	-0.04	-0.07	-0.02	<0.01	-	-	-	-
Pathology prevalence (10%)	0.22	0.12	0.33	<0.01	0.19	0.08	0.30	<0.01
Winter	-	-	-	-	-0.49	-0.95	-0.04	0.03
Summer and Spring	-	-	-	-	0.46	-0.32	1.23	0.25
Variable	Model 3 [†]				Model 4 [†]			
	Estimate	95% Confidence Interval		P	Estimate	95% Confidence Interval		P
Intercept	-2.70	-4.88	-0.53	0.02	-6.18	-7.93	-4.42	<0.01
Age (years)	0.26	-0.39	0.90	0.43	0.31	0.14	0.48	<0.01
Age2 (years)	-0.05	-0.08	-0.02	0.00	-	-	-	-
Pathology prevalence (10%)	0.51	-4.29	5.31	0.84	0.37	0.08	0.65	0.01
Pathology prevalence *Age	1.33	0.08	2.58	0.04	-	-	-	-

^sResults obtained by running random effect negative binomial regression model. Coefficients could be interpreted as Count Ratios after exponentiation.

[†]Results obtained by running random effect logistic regression model. Coefficients could be interpreted as Odds Ratios after exponentiation.

Geographic Information System analysis

Results of the Geographic Information System analysis are presented in the Figure 5. Analysis showed preserved contact between different locations and distribution of water streams that offers favourable conditions for maintaining fluke's life cycle.

Discussion

High efficacy of triclabendazole (up to 100%), albendazole (84%), and rafoxanide (75%) against large American liver fluke (*Fascioloides magna*) has been demonstrated in numerous studies (Foreyt and Todd 1976, Qureshi et al. 1989, Qureshi et al. 1990, Pybus et al. 1991). However, when considering such

high efficacy, one should bear in mind that despite being characterized as 'efficacy against naturally infected', course of treatment was carried out mainly in controlled, captive environment. By contrast, treatment in open, wild habitat is influenced by several factors that can severely minimize its effects. While treating the red deer in Danube wetlands in Austria, Ursprung and colleagues (Ursprung et al. 2006) could not achieve eradication of fascioloidosis, although they managed to reduce significantly the infection. This is an excellent example of successful treatment in wild conditions, which has not been followed that well in other attempts.

Even if the treatment is very successful, usually

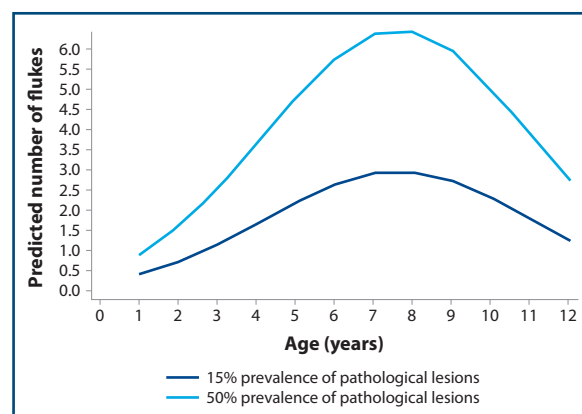


Figure 1. Expected number of flukes in red deer of different age after adjusting for the prevalence of pathological lesions in a specific location. The predictions are based on Model 1 (Table 2). Study period 2007-2012, Spačva area, East Croatia.

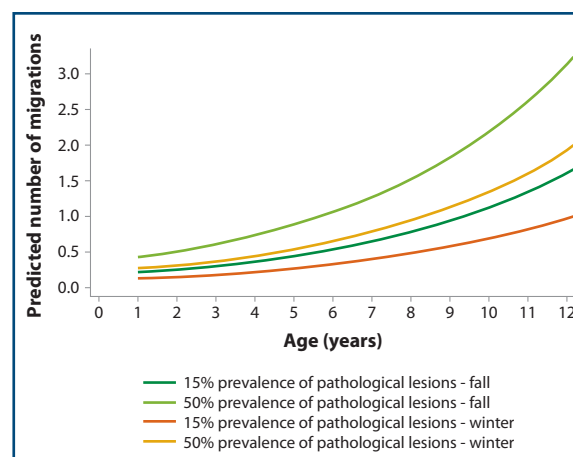


Figure 2. Expected number of fluke migratory stages in livers of red deer of different age after adjusting for the prevalence of pathological lesions in a specific location and the effect of season. The predictions are based on Model 2 (Table 2). Study period 2007-2012, Spačva area, East Croatia.

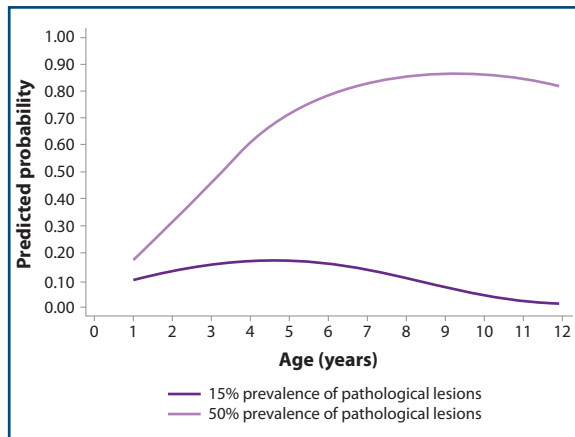


Figure 3. Expected probability of detecting pathological lesions in red deer of different age after adjusting for the prevalence of pathological lesions in a specific location. The predictions are based on Model 3 (Table 2). Study period 2007-2012, Spačva area, East Croatia.

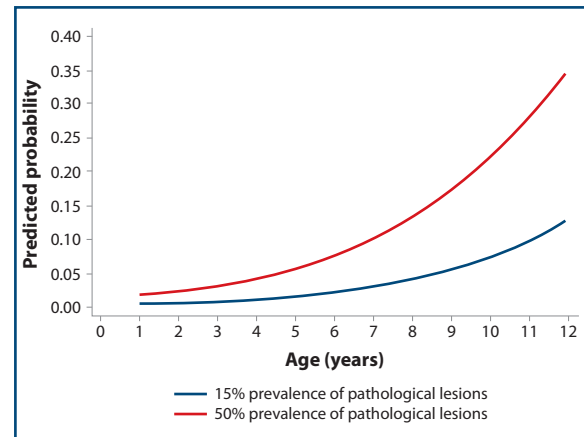


Figure 4. Expected probability of having positive coprological findings in red deer of different age after adjusting for the prevalence of pathological lesions in a specific location. The predictions are based on Model 4 (Table 2). Study period 2007-2012, Spačva area, East Croatia.

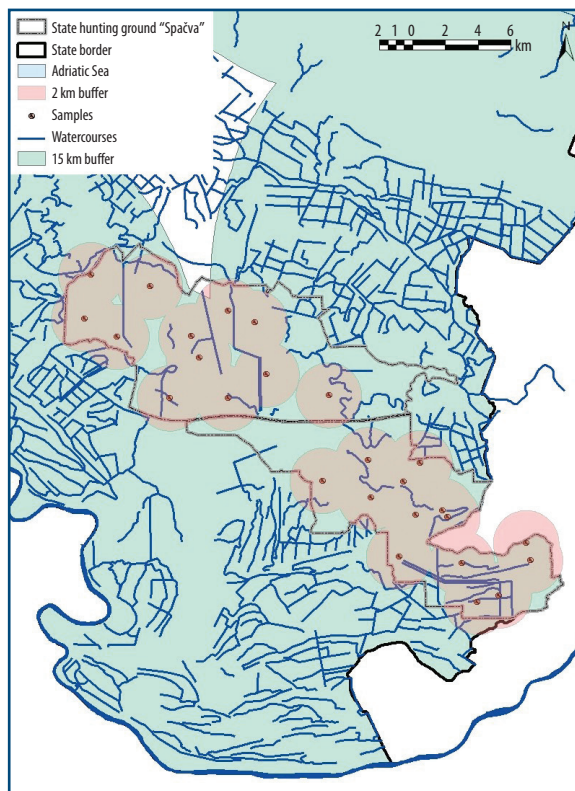


Figure 5. Spačva basin, East Croatia. Points show feeding stations and places where majority of deer reside. Buffer zone (brown circle around points) represent 2 km diameter as potential area used by hinds and calves. Green zone represent an area up to 15 km from state borders as potential area reached by males during horizontal migrations. Blue lines are water streams. Brown line represent hunting ground border.

it is only for a limited time period as conditions of open habitat permits reintroduction of parasite into population under DCP as well as its maintenance in suitable environment. Indeed, recently, there have been indications of recovery of *F. magna*

infections in Austria, based on evidences obtained by screening their intermediate host, namely snail *Galba truncatula* (Haider et al. 2012). This potential of constant introduction of flukes is even more emphasized in the example of Spačva forest basin due to its vast area (approximately 51,000 ha, including the area located in Serbia), especially if keeping in mind that DCP measures were applied only over half of this area (area of the hunting ground XIV/11 "Spačva"). Therefore, we applied epidemiological methods to understand disease dynamics and ecology at population level. As expected, our results indicate that higher prevalence (burden of infection) will result in the earlier infection of deer. This is clearly visible from the fact that prevalence in the second year of life is already around 30%. Interestingly, large liver fluke *Fasciola hepatica* was detected only in 1 case, as a concurrent infection with *F. magna* (Konjević et al. 2011). Also, increasing of infection with age, regardless of the sex of the animal, is present and significant. This is different from our expectations, as we expected that old males would be more important for maintenance and spreading of disease, especially when potential horizontal migrations of males during the rutting season are taken into account (Kleveland 2007). The fact that we did not find correlation between severity of infection and location can be explained by the favourable environmental conditions for flukes and close proximity of different deer groups on the entire hunting ground (Figure 5), as well as with high prevalence rate of up to 46.39% (when fawns are excluded). Since treatment is carried out in open habitat conditions, certain peculiarities, which are not present in captive conditions, must be taken into account. These include influence of resident males and their potential migrations within the analysed area, and the possibility that

some deer would not visit feeding stations or would not consume medicated feed due to the herd hierarchy. Such animals represent constant source of infection and minimize influence of herd location on pathology (we expected higher prevalence close to state borders due to lack of DCP). From the analysis of environmental factors, it is clear that preservation of flukes and maintenance of their life cycles is enabled by high density of water streams. Furthermore, occasional flooding enables the spreading of flukes to various parts of the hunting ground. Similarly, in the example of liver fluke *Fasciola hepatica*, positive correlation between infection rate and humidity and marsh lowland terrains was confirmed (Selemetas and De Waal 2015, Vanderwaal et al. 2015).

Finally, the findings of the present study suggest that drug application to control *F. magna* infections

in wild deer can lead to reduction of infection, but fails to eradicate the disease or to be effective in the long-term after the end of the treatment - especially if the treatment is not conducted on surrounding deer populations. In the case of Spačva basin, all observed data implies that the whole hunting ground can be regarded as 1 epidemiological unit.

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