DE GRUYTER

DOI 10.1515/helm-2017-0035

HELMINTHOLOGIA, 54, 4: 307 – 313, 2017

Observations on autochtonous liver flukes in wild ruminants in Slovakia

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Article info

Received January 10, 2017
Accepted March 28, 2017

Summary

The aim of the study was monitoring of liver flukes in wild ruminants including red deer (*Cervus elaphus*), roe deer (*Capreolus capreolus*), fallow deer (*Dama dama*) and mouflon (*Ovis musimon*) in selected regions of Slovak Republic. Between 2014 – 2016 we examined 782 faecal samples from selected wild ruminants using coprological techniques and serological methods (ELISA detection of *F. hepatica* coproantigens). None of the samples was positive for the presence of *Fasciola hepatica*, 5.89 % of faecal samples were positive for *Dicrocoelium dendriticum*. Higher prevalence was recorded in mouflon (30.83 %), lower in red deer (1.49 %). *D. dendriticum* infection was not determined in fallow deer and roe deer. The seasonal distribution of dicrocoeliosis indicated a highest prevalence in autumn. Significantly higher prevalence was recorded in fenced rearing when compared with open hunting grounds, suggesting that animal agglomeration, constant use of the same areas and possible stress are the main risk factors. Parasitological examination of livers of hunted wild ruminants revealed dicrocoeliosis in mouflon.
Keywords: liver flukes; wild ruminants; *Fasciola hepatica*; *Dicrocoelium dendriticum;* Slovakia

Introduction

In the past 60 years, climate and global changes have contributed to the spread of various diseases of domestic and wild animals. These changes affect also the snail-borne helminthiases, which are strongly dependent on the environment (Otranto & Traversa, 2003; Mas-Coma *et al*., 2005).

In Slovakia, wild ruminants are often infected by a wide spectrum of parasites, including important autochthonous snail-borne helminthes from the *Fasciola* and *Dicrocoelium* genera (Oberhauserová, 2012; Forte *et al*., 2012; Truchanová *et al*., 2015). Such liver flukes are further important because of possible zoonotic potential. *Fasciola hepatica*, “the common liver fluke”, is found in a wide range of species of grazing animals, with the global geographical distribution (Arias *et al*., 2013; Mas-Coma *et al*., 2005; Rojo-Vázquez *et al*., 2012). The incidence of liver fluke is associated with high rainfall and its prevalence is higher in years with rainy summer (Ollerenshaw, 1959). In a definitive host, adults occur in the bile ducts of the liver. Natural infections, found mainly in domestic ruminants, are accompanied with significant clinical signs, including weight loss, yellowish and pale conjunctivae, abdominal pain, diarrhoea, and sudden death (Rojo-Vásquez *et al*., 2012). Even though *F. hepatica* infections occur in domestic ruminants it is not frequently reported in wildlife (Price, 1953; Foreyt & Todd, 1972; Prestwood *et al*., 1975). Experimental infections were described in red deer and roe deer (Barth & Schaich, 1973).

The presence of this parasite in wild ruminants is generally not associated with significant clinical signs or evident pathology. From the veterinary and breeding point of view, *F. hepatica* is of great importance, as it causes huge economic losses in livestock. Fas-

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ciolosis is considered to be an emerging zoonosis (Mas-Coma *et al*., 2005; Rojo-Vázquez *et al*., 2012), known in several regions of the world. Human infections are associated with the consumption of vegetation contaminated with viable metacercariae. Most cases have been found accidentally, during a surgery or an autopsy (Samuel *et al*., 2001; Mas-Coma *et al*., 2005; Špakulová, 2009; Pozio, 2008). Infections with one or two flukes may result in severe reactions difficult to treat. Clinical signs can occur when the larvae are migrating to and through the liver (Samuel *et al*., 2001; Mas-Coma *et al*., 2005; Špakulová, 2009).

Dicrocoelium dendriticum, also known as 'lancet fluke', occurs throughout Europe, Asia, North Africa, and North America (Soulsby, 1968). Adult helminths are present in the bile ducts and gall bladders of a wide range of domestic and wild mammals, particularly domestic and wild ruminants, mainly sheep and mouflon. Camelids, equids, rodents, suids, lagomorphs, and primates can also be infected (Mapes, 1951; Rojo-Vázquez *et al*., 2012). Wild cervids, marmots, and lagomorphs have been involved as reservoirs of infection in domestic species (Mapes, 1951; Nilsson, 1971). Their life cycle lasts 6 months involving two intermediate hosts (Rojo- Vázquez *et al*., 2012). The disease is mainly asymptomatic, it can lead to weight loss and reduced milk production (Otranto & Traversa, 2003; Rojo-Vázquez *et al*., 2012). Several cases of verminous hepatitis caused by dicrocoeliosis have been reported in humans (Cengiz *et al*., 2010; Mapes, 1951; Ondriska *et al*., 1989; Sing *et al*., 2008). A zoonotic potential exists due to ingestion of food or water containing infected ants. Eggs of *D. dendriticum* may be present in human faeces even for several days after the ingestion of infected liver, however, these people are not infected (Cengiz *et al*., 2010; Samuel *et al*., 2001).

The occurrence and spreading of liver flukes is affected by the environment and by the presence of suitable intermediate and definitive hosts (Manga-Gonzales *et al*., 2001; Otranto & Traversa, 2003). Two hosts are necessary for *F.hepatica* to complete its life cycle (Mas-Coma *et al*., 2009) while three hosts are required for *D. dendriticum*. *F. hepatica* is found mainly in aquatic and moist environments suitable for the survival of their intermediate hosts (freshwater snails belonging to the *Lymnaeidae* family*),* a principal intermediate host of the common liver fluke is the cosmopolitan snail *Lymnaea (Galba) truncatula* (Rojo-Vázquez *et al*., 2012; Mas-Coma & Bargues, 1997; Mas-Coma *et al*., 2005). By contrast, the habitat of the small liver fluke *Dicrocoelium dendriticum* is in dry lowlands or mountain pastures with favourable conditions for the development of terrestrial snails and ants (Mas-Coma *et al*., 2005; Otranto & Traversa, 2003; Sandoval *et al*., 2013; Manga-Gonzáles *et al*., 2001).

Material and Methods

Study area

The study was conducted in 3 selected regions (Žilina, Prešov, Košice) out of total 8 regions. Žilina region, the third largest region in the Slovak Republic, expanding on 6,808.4 km^2, is situated in the north- western part of Slovakia. More than a half of the territory (51 %) is occupied by natural areas of different protection level. The surface of the area is mostly mountainous with relatively high average altitude. Moderately warm and cold humid climatic area with lowest temperatures. Prešov region is the second largest region in Slovakia with the area of 8,974.5 km^2. The territory is located in the north-eastern part of Slovakia. The altitude of this area is from 105 m to 2,655 m above the sea level. This rugged territory has mostly mountain character with rich water sources and many meadows and pastures. Košice region covers the area of 6,755 km^2. This largely flat area in southeastern Slovakia extends from 94 m to 1,476 m above the sea level. The north-west territory is characterized by mountains with cold climate and the south is characterized by lowland with moderately climate zone. The warmest area is The East Slovak Lowland, one of the flood plain areas. In all regions there are mixed pastures – cattle, sheep and wild ruminants. Forests, scrublands and fields are shared by wild animals and extensively reared domestic ruminants.

Samples Collection

In the period between 2014 and 2016 faecal samples and livers were obtained from wild ruminants. The total number of faecal samples was 782, from which 624 samples came from the open hunting grounds and 158 samples from fenced rearings. We examined 604 red deer (*Cervus elaphus*), 48 roe deer (*Capreolus capreolus*), 10 fallow deer (*Dama dama*) and 120 mouflons (*Ovis musimon*). 159 samples were collected from Žilina region (152 red deer, 7 roe deer), 452 samples from Prešov region (415 red deer, 37 roe deer) and 171 samples from Košice region (37 red deer, 4 roe deer, 10 fallow deer, 120 mouflon).

After collection, each faecal sample was divided into two, the first part was stored at +4 °C until basic coprological examination (within 72 hours) and the second part was stored at -18 °C until coproantigen ELISA analysis.

We also examined 41 livers (28 red deer, 2 roe deer and 11 mouflon livers). Animals were culled during regular game management operations in Prešov and Košice region. 36

Laboratory methods used

1. Coprological examinations

All faecal samples were examined for the presence of liver flukes using the zinc sulfate flotation method (specific gravity of 1.35 g cm^{-3}) and the light microscopy (Garcia & Bruckner, 1997). Also we used standard sedimentation technique and the modified McMaster method according to Sancho (2013). For classification of intensity of infection of was used egg count interpretation according to the McMaster method (10 eggs per gramme – EPG): low infection (less than 100 EPG), moderate (from 100 – 499 EPG), and severe infection (over 499 EPG). For the presence of *D. dendriticum* faecal samples were processed flotation method using solution with high specific gravity (1.35g.cm^{-3}) and examined microscopically

Table 1. Prevalence of *D. dendriticum* and intensity of infection in wild ruminants.

			Coprological examination *(D. dendriticum)*		
Host	No. examined	No. infected (%)	No. (%) animals with EPG		
			<100	100 – 499	> 499
Red deer	604	9 (1.49 %)	8 (1.32)	1 (0.17)	0 (0.00)
Roe deer	48	0 (0.00 %)	0 (0.00)	0 (0.00)	0 (0.00)
Fallow deer	10	0 (0.00 %)	0 (0.00)	0 (0.00)	0 (0.00)
Mouflon	120	37 (30.83 %)	30 (25.00)	7 (5.83)	0 (0.00)

<100 - low infection; 100-499 – moderate infection; > 499 - severe infection

at 100× magnification (Rinaldi *et al*., 2011; Cringoli *et al*., 2004).

2. ELISA Detection of *F. hepatica*

For the presence of *Fasciola hepatica* coproantigens by ELISA was determined by using the commercial kit BIO K 201/2 Fasciola hepatica Elisa Kit (Bio-X Diagnostics, Belgium). The test was realized according to manufacturer´s instructions. The test results were expressed as an optical density (OD). The limit of positivity for the antigen is 0.150. Any sample that yields a difference in optical density that is greater than or equal to 0.150 is considered positive. Any sample that yields a difference in the optical density that is less than 0.150 is considered negative.

3. Parasitological analysis of the livers

All recieved livers were examined for the presence of liver flukes. The livers have been examined visually and by palpation first, than the bile ducts were opened. The livers were cut into thick slices (10 – 20 mm) and left incubate in warm water for 1 h. Then they were repeatedly squeezed from end to end and washed in tap water. Sediment was sieved and flukes were examined microscopically and classified.

Statistical Analysis

Basic descriptive statistics were used for the analysis of the obtained results. Statistical significance was established when p value < 0.05. Relative risks (RR) and their 95 percent confidence intervals (95 % CI) were estimated for fenced rearing and open hunting grounds.

Results

In the period between 2014 and 2016, 782 faecal samples from wild ruminants were examined in Slovakia for the presence of autochthonous liver flukes *Fasciola hepatica* and *Dicrocoelium dendriticum*.

None of the faecal and liver samples was found positive for the presence of *F. hepatica* eggs and coproantigen.

In areas with the lancet fluke findings in domestic ruminants, we detected infections also in wild ruminants. The results are summarised in Table 1. In 46 out of 782 faecal samples from wild ruminants we isolated characteristic *D. dendriticum* eggs, with the overall prevalence of 5.89 %. Egg counts, expressed in our paper as eggs per gram of faeces (EPG), ranged between 10 and 350, the average was 53 EPG for mouflons. The total EPG in red deer was 10 – 120, the average was 27 EPG. The highest prevalence was observed in mouflons, followed by red deer (30.83 % and 1.49 %, respectively). No positive samples were detected in roe deer and fallow deer. The majority of positive samples were from mouflons (37/46), significantly less positive samples were from red deer (9/46). Positive samples came from 2 out of 3 investigated regions (Prešov and Košice Regions).

The highest prevalence rates were observed in the Košice Region, in the south-eastern part of the country (overall prevalence of 22.22 %), significantly lower prevalence was found in the Prešov Region (1.77 %), and no prevalence was observed in the Žilina Region, these regions are located in more northern areas of Slovakia.

Table 2. Comparison of relative risk of *D. dendriticum* in intensive rearing and free hunting grounds

Free hunting ground		**Intensive rearings**		**Total**		**Relative risk (95%CI)**	**P- value**
N	**P (%)**	**N**	**P (%)**	**N**	**P (%)**		
624	8 (1.28)	158	38 (24.05)	782	46 (7.37)	0.05 (0.03 – 0.01)	**0.001**

N – number of examined samples; P – positive samples (prevalence %)

The increase in *D. dendriticum* prevalence in 2015 and 2016 was observed (9.56 % and 5.20 %, respectively), in comparison with 2014 (1.22 %). The main egg excretion period for the prevalence of *D. dendriticum* in wild ruminants was autumn (9.23 %), as compared to spring, summer and winter seasons (the mean prevalence was 0 %, 5.06 %, and 4.98 %, respectively) (p > 0.001).
Significantly higher *D. dendriticum* prevalence was observed in wild ruminants kept in fenced rearing (24.05 %), as compared to animals from open hunting grounds (1.28 %) (Table 2).
Three mouflon livers, out of 41 examined, were positive for the presence of *D. dendriticum* worms. In these animals, no clinical signs were observed. The total number of parasites in liver varied between 8 and 49 flukes with average fluke burden of 26. 15

Discussion

Previous studies indicate that trematodes, in larval stages parasitizing in invertebrates, may be largely more susceptible to climate changes than those parasites in which such part of the life cycle is absent or reduced to the minimum (Mas-Coma *et al*., 2008). Environmental conditions in the three selected regions of Slovakia are favourable for the liver fluke development, in the northern regions they are suitable for *Fasciola hepatica* and in the southern regions for *Dicrocoelium dendriticum*.
F. hepatica is found in domestic ruminants and many wild animal species all over the world (Rojo- Vázquez *et al*., 2012). Recent reports indicate increasing prevalence of *F. hepatica* in European countries located further north as well as the presence of metacercariae on pastures also in winter, thus, animals are exposed to *F. hepatica* during a long period. This situation has been known for several decades now, mainly in southern European countries (Rojo-Vázquez *et al*., 2012).
In the present study, none of the faecal and liver samples was found positive for the presence of *F. hepatica* eggs and coproantigen, despite some findings in domestic ruminants, especially in the northern regions of Slovakia and on pastures shared with wild ruminants (personal data; Oberhauserová, 2012). According to earlier statistics from district abattoirs in Slovakia, fasciolosis resulted in the condemnation of 23.1 % of cattle livers and 35.8 % of sheep livers during the period of 1954 – 1955 (Podhájecký, 1958). Infections in domestic ruminants were also observed in neighbouring countries – the Czech Republic, Poland, and Ukraine (Chroust, 2006; Kiziewicz, 2013; Michalski & Rimaniuk, 2000; Uradziński & Radkowski, 1992; Brodovsky, 2015). Insufficient data are available with regard to fasciolosis in wild ruminants in Slovakia – in 2011, the presence of *F. hepatica* parasites was detected in European bisons in the Poloniny National Park (4 out of 25 examined animals were positive – the prevalence of 16 %, EPG 50). No presence of common liver fluke was recorded in bisons in 2012 (Forte *et al*., 2012). No case of fasciolosis in red deer, roe deer, fallow deer, and mouflons has been detected in Slovakia for the last 8 years (Oberhauserová, 2012; Truchanová *et al*., 2015). The presence of fasciolosis in wild ruminants in the Czech Republic has been greatly reduced by intensive therapies, particularly in domestic ruminants, (Chroust & Forejtek, 2010). Earlier research and monitoring has not detected any presence of *F. hepatica* in roe deer in the Czech Republic – despite heavy infections in cattle (Vetýška, 1980; Dyk & Chroust, 1974; Koždoň, 1975). In recent years, common liver fluke occurs especially in the north-western and northern parts of the country with a low prevalence and intensity (Chroust & Forejtek, 2010). Investigations in various areas of neighbouring Poland revealed the decline in the prevalence of fasciolosis in domestic as well as wild ruminants, compared to the 1990s (Uradziński & Radkowski,1992; Kiziewicz, 2013).
The results of field and experimental investigations indicate that cervids are not significant reservoirs of *F. hepatica.* Limited infections may be caused by ecological barriers that limit the exposure and by some innate resistance to infections (Samuel *et al*., 2001).
Over the last decades, an increasing number of dicrocoeliosis cases has been reported worldwide in lowlands and mountain pastures, with certain economic as well as health consequences, especially in the case of extensive livestock breeding (Sandoval *et al*., 2013). In many European countries, the prevalence in sheep herds amounts to as much as 100 % (Manga-Gonzáles *et al*., 1991). Such increase may be explained by the implementation of efficient control measures against other trematodes and affected by warmer springs and summers, extending dry habitats needed for *D. dendriticum* intermediate hosts (Otranto & Traversa 2002; Taylor 2012; Rojo-Vázquez *et al*., 2012).
The comparison between our results and the results in domestic ruminants obtained by Konigová *et al*., (2016), i.e., the EPG in sheep of 10 – 240 and the average of 120, and the results obtained by Gonzáles-Lanza *et al*., (1993), i.e., the EPG in cattle of 10 – 10 000 and the average of 42, shows that the parasitic burden was lower in wild ruminants (Table 1). Research in the Poloniny National Park revealed the presence of *D. dendriticum* eggs in European bisons, with the average EPG of 50 (Forte *et al*., 2012).
In the last years, there is a lack of data on the prevalence of dicrocoeliosis in domestic and wild ruminants in Slovakia. Previous studies revealed *D. dendriticum* infections in mouflons (prevalence of 3.74 %) (Oberhauserová *et al*., 2012), European bisons (prevalence of 16 %) (Forte *et al*., 2012), and chamois (Ciberej *et al*., 1997). In 2016, some authors observed a high incidence of dicrocoeliosis in selected sheep farms in Slovakia (Konigová *et al*., 2016).
The importance of dicrocoeliosis in ruminants has also been investigated by Ducháček and Lamka (2003) who reported the prevalence of 54 % in Slovakia in years 1984 – 1986 and 21 % in Hungary in 1993. The prevalence of as much as 32 % was detected in Germany in 1975 and between 27 % and 63 % in the north of Spain in years 1991 – 1993. In the Czech Republic, there are not any problems with dicrocoeliosis in cattle, it occurs mainly in sheep and mouflons, occasional infections were observed in roe deers (Chroust, 2006).

The present study indicates a seasonal distribution – the main egg excretion period for the prevalence of *D. dendriticum* in wild ruminants was autumn, as compared to spring, summer and winter seasons. The seasonal variations have also been confirmed by other authors, with *D.dendriticum* eggs continuously present in faeces throughout the year, but the highest values were observed in the cold season (Sandoval *et al*., 2013), at the end of autumn and in winter (Manga-Gonzales *et al*., 2010; Gonzales-Lanza *et al*., 1993; Jithendran & Bhat, 1996).

In the last two years, we observed the increase in the number of *D.dendriticum* cases. With regard to the climatic conditions in Slovakia (2014 and 2015 were the warmest years in the history of meteorological measurements, 2016 was above the long-term average).

Significantly higher *D. dendriticum* prevalence was recorded in fenced rearings when compared with open hunting grounds According to Otranto and Traversa (2002), stress-inducing factors, such as animal capture and transport, proved to increase egg production, probably caused by immune depression in wild animals. Another fact is that the eggs remain on pastures for as much as 20 months (Otranto & Traversa; 2002).

Dicrocoelium dendriticum is often underestimated by vets and researchers due to frequent subclinical course of infection (Otranto & Traversa, 2003), or clinical signs are not pathognomic, i.e. animals can develop oedemas, icterus, anaemia and weight loss. Rojo-Vázquez *et al*., (1981) confirm that parasitic burden by less than 1,000 flukes does not result in clinical manifestation.

An average fluke burden in wild ruminants is lower than indicated by other authors for domestic ruminants (30 – 2,063; 60 – 437; 12 – 1,230 in sheep, 8 – 1,208 in goats – Manga-Gonzáles & Gonzáles-Lanza, 2005; Jithendran & Bhat, 1996).

Several authors confirm that cross infections transmitted from livestock to wild ruminants and vice versa can occur in common grazing pastures (Arias *et al*., 2013; Rojo-Vázquez *et al*., 2012); despite the fact that the epidemiological significance of the occurrence and fluke transmission from wild to domestic ruminants has not been sufficiently clarified (Rojo-Vázquez *et al*., 2012). The contamination of pastures and dispersal of *D. dendriticum* eggs can be caused by wild cervids which are thus able to play two roles – as reservoirs of pathogens and as an indicator of domestic animal diseases (Arias *et al*., 2013; Boray, 1985; Otranto & Traversa, 2002; Taylor, 2012). The limited number of worms and also lower egg counts in wild ruminants suggest inherent resistance to infections (Samuel *et al*., 2001).

The strong dependence of liver flukes incidence on weather factors indicates that climate change may have a remarkable influence on the future evolution of these diseases.

As the wildlife can represent a threat to domestic animals, monitoring of these diseases should to be considered to be as important as in domestic animals. High population density in fenced rearing, as well as the autumn/winter season on open hunting grounds, represent stress-inducing factors for animals which can significantly affect incidence and severity of diseases. Correct dehelminthization, in terms of timing and dosage, will play an essential role in the efforts to solve the issues of antihelmintic resistance and reduction of economic impact of these parasites, particularly in fenced rearings.

Acknowledgements

This research was supported by the Science Grant Agency VEGA No. 1/0791/14

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