

Parasitic infections of the European brown hare (*Lepus europaeus* Pallas, 1778) in south-western Slovakia

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Summary

A total of 74 European brown hares (*Lepus europaeus* Pallas, 1778), hunted during the winter seasons of 2006 and 2007, were examined by dissection for the presence of helminths and coprologically for protozoa. The animals came from five districts with a high population density of this species. Our results revealed 54.5 % of specimens being infected with one or more helminth species and a high prevalence of eimeriid coccidia (91.89 %). The most prevalent helminth species was *Trichuris leporis* (55.41 %). Lower prevalence was found for *Passalurus ambiguus* (12.16 %) and *Trichostrongylus retortaeformis* (6.76 %). The intensity of infection was low for all parasite species. As for coccidia, *Eimeria semisculpta* (74.35 %) and *E. leporis* (61.54%) were recorded in all districts. Other coccidia showed lower prevalence rates: *E. robertsoni* (15.38 %), *E. europaea* (12.82 %), *E. babatrica* (12.82 %), *E. hungarica* (5.13 %) and *E. towsendi* (2.56 %), occurring only in some districts. The highest infection rate was observed in *E. semisculpta*, 7657.8 oocysts per gram of faeces (OPG). The potential effect of protozoan infection on hare mortality is discussed.

Keywords: hares; eimeriid coccidia; helminths; Slovakia

Introduction

European brown hare *Lepus europaeus* Pallas, 1778 is an important part of the wildlife fauna. This typical inhabitant of agrarian landscapes prefers agricultural land in the southwest and southeast of Slovakia, where the population is the most abundant. Hare numbers were high until 1974; annually up to 354 000 animals were hunted. In the 1970's a steep decline in the hare population was recorded and in 1982 only 10 % of the above number was hunted. In the following years, the population dynamics was unstable and hare harvest was very low (Slamečka *et al.*, 1997). A similar decreasing trend in hare numbers has been noted

throughout Europe for the past thirty years (Marboutin *et al.*, 2003). There are many probable reasons for fluctuations in the hare population in Europe; however, deterioration of animal health strongly influenced by anthropization of landscape seems to be the key factor.

Hares suffer considerably from predation, particularly by foxes (Newey *et al.*, 2007; Romig *et al.*, 2007; Di Cerbo *et al.*, 2008; Miterpáková *et al.*, 2009), and the decline was accompanied by a growth in predators in recent years. The population density may also be influenced by different diseases, some of them occurring only locally. Parasitic diseases are also considered to be a regulative factor of hare population (Alzaga *et al.*, 2009).

The parasitostatus in European brown hare in Slovakia and neighbouring countries was surveyed particularly in the 50's and 60's of the last century and the results are summarised by Nickel and Gottwald (1979). The authors indicate a diverse species composition of protozoa and helminths. In the first group, *Eimeria semisculpta*, *E. europaea* and *E. leporis* predominated. In localities with endemic occurrence of *Fasciola hepatica* and *Dicrocelium dendriticum* these helminths were sporadically also found in the gall bladder and common bile ducts of hares. In the intestines adult tapeworms were observed sporadically, with *Mosgovaya pectinata* being the most prevalent species. Out of other helminth species infecting the digestive tract, *Graphidium strigosum* in stomach, *Trichostrongylus retortaeformis*, *Trichuris leporis* and *Passalurus ambiguus* in intestines were most frequent. The most pathogenic species are *G. strigosum* in the stomach and *Protostrongylus commutatus* in the lungs (Kotrlá, 1984).

The records on the parasitic status of the European brown hare in Europe include a wide spectrum of parasitic species. Soveri and Valtonen (1983) found 2 coccidian and 3 helminth species in Finland, whereas Allgöwer (1992) detected 6 coccidian and 5 helminth taxa in Schleswig-Holstein. Pakandl (1990) referred to the occurrence of 9

coccidian species in different parts of the former Czechoslovakia with the prevalence in hares ranging from 1.3 % up to 74.8 %. Aoutil *et al.* (2005) recorded infestation with new eimeriid coccidia species in hares from France. Lungmayr *et al.* (2008) found parasites (both helminths and protozoa) in 100 % of hares originating from Belgium and Austria.

In Slovakia, as well as in the former Czechoslovakia, the parasitostatus of hare was very similar to those in neighbouring countries 50 years ago. Ryšavý (1954) referred to the presence of 8 eimeriid coccidia species; Kotrlá (1984) mentioned 7 most prevalent of them. Dobšínský (1955) found 5 helminth taxa in the area of eastern Slovakia. Ašmera *et al.* (1965) detected 5 species of eimeriid coccidia and 6 helminth taxa in hares from northern Moravia. In the areas of the Tatras National Park, Mituch (1970) identified 8 helminth species. The data on parasitic fauna composition obtained by coprological examination of hares from the area of south-western Slovakia (Jurčík *et al.*, 1995, 2007) revealed a high prevalence of coccidiosis caused by 8 species. The oocysts of *Eimeria semisculpta* were the most frequent (89.3%) and other species were present in one third of samples. The long-term high prevalence rates of *Trichostrongylus* spp. and *Trichuris leporis* spp. recorded were also surprising. This paper describes the infestation with helminths detected by means of stomach and intestines dissection and quantitative occurrence of coccidian oocysts in faeces of brown hares hunted within two winter seasons in five south-western districts of Slovakia.

Material and Methods

A total of 74 European brown hares were shot in seven sites of five districts (Trnava, Senec, Galanta, Šaľa and Dunajská Streda) during the winter seasons of 2006/2007 and 2007/2008. The eye lenses were removed in order to estimate their age (Hell, 1972). The eyeball was removed

with blunt scissors, placed into a sample case with 4 % formaldehyde and allowed to fix for 14 days. The isolated lenses were then placed on a watch glass dried in 110 °C for 24 hours and weighed. According to the age, hares were divided into two groups: the young and sub-adult hares, and the adults. The population dynamics in individual years were evaluated according to the proportion of young animals in game bags as a percentage of the reproduction coefficient R (the ratio of young animals per adults), and the reproduction factor r (the number of young hares per adult female) (Slamečka *et al.*, 1997). The stomach and intestines were removed from all 74 hares, and the liver and lungs from six of them. The organs were then stored at -20 °C until examination. Following dissection, helminths were counted and fixed in 70 % ethanol, or Barbegal solution.

Two grams of faecal samples were removed from the rectum, mixed with 15 ml of 2.5 % potassium dichromate solution and left for sporulation at a room temperature. *Eimeria* species were determined according to Černá (1983), Pakandl (1990) and Aoutil (2005). The number of oocysts per gram of faeces (OPG) was defined by the quantitative McMaster technique as described in the Manual of Veterinary Parasitology Techniques (Anonymous, 1986).

Differences in prevalence and intensity of infection were determined using Chi-square tests. Values were considered significant at $P < 0.05$.

Results

The results of the dissection, coprological examinations and data on the presence of endoparasites in hares are given in Table 1. As much as 57.1 % of the animals were juveniles and sub-adults, with females comprising 76.5 %. The data refer to a high occurrence of endoparasites in the digestive system, as the prevalence rates reached 100 % in several localities. The differences in hare infestation were not

Table 1. Parasitostatus of *Lepus europaeus* in south-western Slovakia in 2006 and 2007

District	Locality	Year	No. of examined	Protozoa		Helminths		
				Infected	Prevalence (%)	Infected	Prevalence (%)	
Trnava	Trnava	2006	9	9	100.0	5	55.56	
		2007	5	5	100.0	2	40.0	
Senec	Čataj	2007	6	6	100.0	2	33.3	
	Žihárec	2006	7	6	85.71	5	71.43	
Šaľa	Diakovce	2006	6	5	83.33	4	66.67	
		2007	6	6	100.0	6	100.0	
	Vlčany	2006	9	8	88.89	5	55.56	
D. Streda	Lehnice	2006	10	8	80.0	5	50.0	
		2007	4	3	75.0	0	0	
	Č. Radvaň	2006	5	5	100.0	3	60.0	
		2007						
		2006	35	31	88.57	19	54.29	
TOTAL		2007	39	37	94.87	21	53.85	
		2006 – 2007	74	68	91.89	40	54.05	

Table 2: Species composition of *Eimeria* spp. and number of oocysts in 1 g of faeces (OPG) in *Lepus europaeus*

District	Locality	No. of examined	<i>E. semisulcata</i>			<i>E. leporis</i>			<i>E. europea</i>			<i>E. robertsoni</i>			<i>E. habatica</i>			<i>E. hungarica</i>			<i>E. townsendi</i>			
			Inf.	OPG	Inf.	OPG	Inf.	OPG	Inf.	OPG	Inf.	OPG	Inf.	OPG	Inf.	OPG	Inf.	OPG	Inf.	OPG	Inf.	OPG	Inf.	OPG
Trnava	Trnava	5	4	527	3	317	1	50	2	150	1	200	-	-	-	-	-	-	-	-	-	-	-	-
Senec	Čataj	6	3	1850	3	350	2	175	1	150	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Galanta	Žiháreč	7	7	3914	4	875	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Šal'a	Vlčany	6	6	34742	5	2690	-	-	-	1	1000	1	300	2	175	-	-	-	-	-	-	-	-	-
D. Streda	Lehnice	10	7	2864	6	1175	1	50	2	75	3	16433	-	-	-	-	-	-	-	-	-	-	-	-
Č. Radvaň		5	2	2050	3	217	1	150	-	-	-	-	-	-	-	-	-	-	-	-	1	500	-	-
		Prevalence (%)	74.35			61.54			12.82			15.38			12.82			5.13			2.56			
TOTAL		OPG ± S.D.	7657.8 ± 12155.7			937.2 ± 845.6			106.3 ± 56.9			343.8 ± 380.1			5644.4 ± 7628.9			-			-			

Table 3. Helminth species composition in *Lepus europaeus*

District	Locality	Year	<i>Trichuris leporis</i>			<i>Trichostrongylus retortaeformis</i>		<i>Passalurus ambiguus</i>			
			No. of examined	Infected	Prevalence (%)	Infected	Prevalence (%)	Infected	Prevalence (%)		
Trnava	Trnava	2006	9	5	55.56	-	-	-	-		
		2007	5	1	20.0	-	-	1	20.0		
Senec	Čataj	2007	6	1	16.67	-	-	2	33.3		
		2006	7	3	42.86	3	42.86	2	28.57		
Galanta	Žihárec	2007	7	4	57.14	-	-	1	14.29		
		2006	6	4	66.67	-	-	-	-		
Šaľa	Diakovce	2007	6	6	100.0	-	-	2	33.3		
		2006	6	6	100.0	-	-	-	-		
D. Streda	Lehnice	2006	9	5	55.56	2	22.2	1	11.1		
		2007	10	5	50.0	-	-	-	-		
	Č. Radvaň	2006	4	-	-	-	-	-	-		
		2007	5	3	60.0	-	-	-	-		
			2006	35	21	60.0	5	14.29	3	8.57	
TOTAL			2007	39	20	51.28	-	-	6	15.38	
			2006 – 2007	74	41	55.41	5	6.76	9	12.16	

significant between the two years. Eimeriid coccidia were the major parasites with the prevalence rate of 91.89 %. Helminths were detected in 54.05 % of examined animals. In hunted hares, seven coccidia species were determined. Their composition, as well as oocysts output, varied greatly within the sites examined (Table 2). Hares from all localities harboured *E. semisculpta*, with the prevalence rate of 74.35 % and the high OPG values of 7 657.8 oocysts. *E. leporis* was detected in 61.54 % of the animals, but the OPG value was only 937.2 oocysts. Other coccidian species were present only in 2.56 – 15.38 % of the hares, either in the most of the districts (*E. europea*, *E. robertsoni*), or sporadically (*E. babatICA*, *E. hungarica*

and *E. towsendi*). Except for *E. babatICA*, reaching as much as 16 433 OPG in the locality of Lehnice, the mean OPG values were very low.

The helminth species composition of the digestive systems in hares was rather limited (Table 3). *Trichuris leporis* was the only species detected in all districts with the mean of 55.41 % of infected specimens. The prevalence varied from 16.67 % in the Čataj locality up to 100 % in Vlčany locality. Out of 74 hares, only five animals (6.76 %) harboured *Trichostrongylus retortaeformis*. *Passalurus ambiguus* was present in nine hares (12.16 %). No significant difference was found in the numbers of infected individuals between years of examination. The helminth burden

Table 4. Change to the correlation between coccidia oocysts numbers and population growth of *Lepus europaeus*

District	Locality	Indicator of hare population growth				No. of coccidia oocysts (OPG)	
		Proportion of young animals (%)	Reproduction coefficient (R)	Reproduction factor (r)	<i>E. semisculpta</i>	<i>E. leporis</i>	
Trnava	Trnava	49.53	0.98	1.82	527	317	
Senec	Čataj	29.63	0.42	1.6	1850	350	
Galanta	Žihárec	38.64	0.62	1.0	3914	875	
Šaľa	Vlčany	12.50	0.14	0.22	34742	2690	
Dunajská Streda	Lehnice	20.0	0.26	0.55	2864	1175	
	Čiližská Radvaň	35.42	0.54	0.94	2050	217	
Corellation Coefficient (r±) with OPG <i>E. semisculpta</i>		-0.27	-0.62	-0.68			
Corellation Coefficient (r±) with OPG <i>E. leporis</i>		-0.79	-0.70	-0.81			

was low (1 to 3 specimens) in all positive animals and no patho-morphological changes related to infection were observed. All the lungs and livers examined had no helminths or their larval stages.

Evaluation of hares from six localities according to their age and gender enabled us to determine the hare population status and evaluate population growth in relation to the number of oocysts of the eimeriid coccidia species that were determined in all localities (Table 4). Only a weak negative correlation ($r = -0.62$, resp. $r = -0.68$) was observed among the number of *E. semisculpta* oocysts and reproduction coefficient or reproduction factor. However, a high negative correlation ($r = -0.70$ to $r = -0.81$) was found between the number of *E. leporis* oocysts and the indexes of population growth.

Discussion

The population of European brown hare has fluctuated regularly. According to Bakoš and Hell (1981), its population cycle lasts on an average from 10 to 12 years, with a distinct oscillating of the population growth curve both in its upper and lower levels, the most noticeable in a 3 to 6-year intervals.

Partly due to different environmental conditions, the hare population in Slovakia is geographically unevenly distributed. In 2007, the highest spring stock numbers reached 73 510 animals in the Nitra region and 63 230 animals in the Trnava region, probably due to the existence of large farmland areas in this territory. In total, 28 604 hares have been hunted this year in these regions, i.e. 89.9 % of the national production. Per 100 ha, 5.3 animals were hunted in the Trnava region and 3.2 animals in the Nitra region. In the Košice region, it is as low as 0.3 animals per 100 ha, even though the farmland area covers 52.9 % of the landscape. The population growth rates fluctuated rapidly, varying from 35.13 % (expressed as percentage of yearlings in the game bags) in 2006 to 57.42 % in 2007 (Slamečka & Hell, 2009).

Fluctuation of the hare population has been attributed to many factors, in particular to climatic conditions in the relevant year, which greatly influence animal morbidity (Bakoš and Hell, 1981; Slamečka *et al.*, 1997). Unlike other time- and area-limited infections, the agents of parasitic diseases occur permanently in the hare population. They negatively affect particularly animals already weakened by other factors. Leverets are especially vulnerable to several coccidia species that are highly pathogenic, the stomach damaging haematophagous helminth *G. strigosum* and the lungworm *P. commutatus*, which affects the parenchyma of the lungs, leading to secondary bacterial infections resulting in death (Sitko *et al.*, 1970). However, no lungworms were detected in hares from our studied localities, similar to the data of Jurčík *et al.* (2007) during 2004 and 2005.

The present survey revealed high prevalence rates of eimeriid coccidia (91.89 %), whilst helminth prevalence was lower (54.05 %). Differences in prevalence rates be-

tween years were insignificant. The data refer to long-term stable parasite prevalence in the hare population. Similar results were observed by other authors (Ašmera *et al.*, 1965; Pakandl, 1990; Allgöwer, 1992; Jurčík *et al.*, 2007). High coccidia prevalence in hares has probably been due to the predominance of young and sub-adult animals (57.1 %) in the sampling. Two species, *E. semisculpta* and *E. leporis*, were detected in all localities with the high mean prevalence rates (74.35 % resp. 61.54 %). Since young hares are particularly vulnerable to coccidian infections, we analyzed the correlation between the number of oocysts (OPG) and population dynamics in relevant hunting grounds. Our data showed that *E. leporis* has negatively affected the indexes of population growth, thus confirming that this species is more pathogenic than the other (Pellérday *et al.*, 1974; McCulloch *et al.*, 2004). Our findings also confirm the correlation between the age and OPG, and thus potential pathogenicity of coccidia. The highest OPG values in *E. semisculpta* (34742 oocysts) and *E. leporis* (2690 oocysts) were recorded in Vlčany, where only young hares were examined. In the recent study from Poland, three *Eimeria* species reached the highest OPG - *E. europaea* (18756 OPG), *E. leporis* (18381 OPG) and *E. robertsoni* (8294 OPG) (Pilarczyk *et al.*, 2008).

Prevalence rates for other *Eimeria* species were low and they occurred in some localities only in isolated cases. Similar findings on variable occurrence of eimeriid coccidia in hares were also reported by other authors. Gottschalk (1973) recorded the highest prevalence of *E. europaea* (46.7 %) in Germany; Gräfner *et al.* (1967) found in Germany near Schwerin the highest prevalence of *E. semisculpta* (94.3 %) and *E. leporis* (50.0 %) which is consistent with our results from Slovakia. A high prevalence of *E. leporis* was reported by Nickel and Gottwald (1979) near Dresden. Kutzer and Frey (1976) referred to a high prevalence of *E. semisculpta* (52.1 %) and *E. leporis* (47.5 %) in hares from Austria, but *E. robertsoni* were even more prevalent (53.6 %). The latest survey conducted in Austria reported seven coccidian species found in hares (*E. semisculpta*, *E. robertsoni*, *E. leporis*, *E. europaea*, *E. septentrionalis*, *E. stefanskii* and *E. townsendi*) (Lungmayr *et al.*, 2008). Pakandl (1990) identified *E. townsendi* as predominant species (67.1 % – 81.78 %) in the Czech Republic during 1983 – 1985, while *E. leporis* and *E. robertsoni* were less prevalent. Böckeler *et al.* (1994) found *E. semisculpta* as the most frequent coccidia in hares from Schleswig-Holstein (52.0 %), *E. leporis* was less frequent (29.0 %) and *E. robertsoni* prevalence rates were similar (15.0 %) to those found in southern Slovakia (15.38 %). Concerning the helminth fauna of hares, *T. retortaeformis* was the most prevalent species in Austria (Kutzer & Frey, 1976), Poland (Czaplińska *et al.*, 1965), Germany (Nickel & Gottwald, 1979), and the Czech Republic (Ašmera *et al.*, 1965). On the other hand, Pilarczyk *et al.* (2008), in the study performed in Poland, recorded low number of hares infected with this parasite species. Compared with *T. leporis*, lower prevalence of *T. retortaeformis* was reported also in Slovakia by Dobšinský (1955), Mituch (1970)

and Kotrlá (1984). Our data confirm such records, as the *T. leporis* prevalence was higher (55.41 %) than that of *T. retortaeformis* (6.76 %), with an isolated occurrence in both districts.

Previous studies found a very low prevalence of *P. ambiguus* (Nickel & Gottwald, 1979; Böckeler *et al.*, 1994). Also Allgöwer (1992) found its prevalence to be 15 % in hares from the Rhine region in Germany. Contrary to those data, Kotrlá (1984) stated 72 % of hares infected with this species, however with low infection rates.

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