ENDOPARASITES OF EUROPEAN BROWN HARE (*LEPUS EUROPAEUS*) FROM SOUTHERN POLAND BASED ON NECROPSY*

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Abstract

The population of the European brown hare (*Lepus europaeus*) has been declining for the last decades in many European countries, including Poland. The goal of this study was to determine the level of endoparasite infection among hares. In 2007–2010, 83 animals were examined postmortem. The animals were weighed and analysed according to age and sex. During the dissection only the following nematodes were noticed: *Trichostrongylus retortaeformis*, *Strongyloides papillosus*, *Trichuris leporis* and *Passalurus ambiguus* in the intestinal tract and *Protostrongylus pulmonalis* in lungs. Body mass of hares was analysed with a general linear model (GLM) with age, sex, and presence/absence of nematode infection as factors. The proportion of infected and uninfected hares with protozoan coccidia was compared with Fisher exact test for 2×2 contingency tables, whereas the proportion of nematode infection was compared by χ^2 test. There was a significant difference in the proportion of hares infected and not infected by coccidia with the higher proportion of infected juvenile individuals (P=0.010), whereas there was no difference between males and females (P=0.41). The frequencies of hares infected vs. not infected by nematodes did not differ between sex (χ^2=1.89, P=0.168) and age (χ^2=0.0007, P=0.97). The mean body mass of all hares was 4.15 kg±0.40 kg. GLM model conducted for body mass of hares showed that there was a significant difference only between juvenile and adult hares (F=24.225, P=0.000005) and no significant association between the level of endoparasite infection and sex.

Key word: *Lepus europaeus*, body weight, sex, parasites, necropsy

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The European brown hare (*Lepus europaeus*), of the Lagomorpha order, is a valuable game animal. In Poland, apart from this widespread species, mountain hare (*Lepus timidus*) and European rabbit (*Oryctolagus cuniculus*) also occur locally. There has been a visible decline in the number of brown hares during the last 30 years in Poland as well as in other European countries (Dziedzic et al., 2000; Kamieniarz and Panek, 2008; Smith and Johnston, 2008). The reasons behind this decline are attributed to multiple factors and yet there is no evident consent (Smith et al., 2005). Firstly, ecological variables such as predation from red foxes (*Vulpes vulpes*) and diseases have been increasing in recent years. Secondly, abiotic components, which cover climatic conditions, soil conditions and the structure of agricultural areas, can also be attributed to the decline of hares. Additionally, the population decline has also been caused by anthropogenic factors such as chemical soil contamination and changes in land use through intensive agriculture, hunting and poaching, and road and highway traffic as well as genetic factors (Dziedzic et al., 2000; Sadzikowski et al., 2007; Kamieniarz and Panek, 2008; Andersen et al., 2009; Misiorowska and Wasilewski, 2012; Thulin et al., 2012).

Diseases are one of the natural factors of hare mortality and have greatly influenced the animals' decline (Dziedzic et al., 2000; Wibbelt and Frölich, 2005). In the last few years, special attention has been devoted to a viral disease called European brown hare syndrome (EBHS), which was found among hares in many European countries, e.g. Greece (Billinis et al., 2005), England (Duff et al., 1994), Switzerland (Frölich et al., 2001, 2003), Sweden (Gustafsson et al., 1989; Syrjälä et al., 2005), and Poland (Frölich et al., 1996). Apart from viral and bacterial diseases, parasitic infections may also have negative influence on the abundance of hares (Wibbelt and Frölich, 2005; Sadzikowski et al., 2007). The most typical parasites of European brown hare are coccidia from the *Eimeria* genus, flatworms (*Fasciola hepatica*, *Dicrocoelium dendriticum*, *Cittotaenia denticulata*, *Andrya rhopalocephala*, *Mosgovoyia pectinata*, *Taenia pisiformis*), gastrointestinal nematodes (*Graphidium strigosum*, *Trichostrongylus retortaeformis*, *Strongyloides papillosus*, *Passalurus ambiguus*, *Trichuris leporis*), and lungworms (*Protostrongylus pulmonalis*) (Nickel and Gottwald, 1979; Soveri and Valtonen, 1983; Pajerský et al., 1992; Laakkonen et al., 2006).

Even though international literature suggests a tremendous impact of parasite infections on hares, only a few studies in Poland have been conducted on parasites in wild hare populations; moreover, some of the studies took place at least several decades ago (Wieczorkowski, 1968; Kozakiewicz et al., 1982; Gundłach et al., 2004; Pilarczyk et al., 2008). Therefore, the main aim of this study was to update knowledge on the internal parasite species composition, their prevalence and intensity of infection among European brown hare (*Lepus europaeus*) from southern Poland (Kraków area). In addition, we tested if infection by nematodes and coccidia depends on body mass, gender, and age of the hares and how these results relate to findings reported by other authors.

Material and methods

From 2007 to 2010, 83 hares were shot, weighed and subjected to further analyses by hunters from Kraków Province. Digestive tracts and internal organs (lung, liver, spleen, kidney and heart) were collected from the animals. A total of 51 juvenile hares (less than 8 months old) and 32 adults (more than 8 months old) were examined. Among juveniles, there were 24 females and 27 males, while the sex distribution among the adults was 16 females and 16 males. The age of the hares was estimated by the radius and ulna ossification stage examined with a Strohl-tubercle (Pielowski, 1979).

The contents of the stomach, small intestine and large intestine after decantation in black cuvettes (developing trays) were independently examined by eye for parasite specimens. Additionally, the mucus and content of duodenum rinsed in hot water was scraped by a blunt knife and washed through 125 mm sieve. Prepared samples were examined under a binocular microscope for presence of small nematode specimens.

Parasites were collected also from the lungs cutting bronchus lengthwise, after which lung tissue was rinsed and squeezed in saline to find other parasites.

All collected specimens of parasites were preserved in 70% alcohol with 5% glycerol for the clarification. Differentiation of the species was determined under a microscope (magnification 40, 100, and 400x) based on the morphology of the parasites, with descriptions given by Stefański and Żarnowski (1971), Gibbons and Khalil (1982) and Anderson et al. (1982).

Additionally, we applied flotation method and McMaster method (MAFF, 1986) to examine the presence of coccidia in the faeces (n=31). Due to the prolonged freezing of hares' digestive tracts, the sporulation of oocysts was not possible as a method for differential diagnosis of *Eimeria* species.

Based on the necropsy, the prevalence and the intensity of parasite infection were evaluated. The oocyst output of coccidia in 1 g of faeces (OPG) was also calculated, based on coproscopy. Body mass was analysed with a general linear model (GLM) with age, sex, and presence/absence of nematode infection as factors. The proportion of coccidia infected and uninfected with protozoans was compared with Fisher exact test for 2×2 contingency tables, whereas the proportion of nematode infection was compared by χ^2 test. Both statistics were composed in relation to sex and age of hares. Statistical analysis was performed using the Statistica 9.0.

Results

The presence of coccidia from the *Eimeria* genus was noticed in the examined faeces of the hares. The prevalence of *Eimeria* infection in young hares was relatively greater than in the old ones, 50% and 6.7% respectively (Table 1). The analysis with the use of the Fisher exact test confirmed that there was a significant difference in the proportion of hares infected and not infected by coccidia with the higher proportion of infected juvenile individuals (P=0.010), whereas there was no difference between males and females (P=0.41).

Table 1. Prevalence (%) and mean intensity of infection ± SD (min – max) in different groups of hares. N – number of hares tested for coccidian infection, N_1 – number of hares tested for nematode infection

Hare characteristics Number of tested individuals	Parasite species					
	Eimeria sp[1]	*Strongyloides papillosus*	*Trichostrongylus retortaeformis*	*Trichocephalus leporis*	*Passalurus ambiguus*	*Protostrongylus pulmonalis*
Juveniles N=16 N_1=51	50.0% 1596±1699.9 (20–4200)	-	25.5% 18±32.8 (1–118)	39.2% 26±21.8 (1–65)	3.9% 65±68.6 (17–114)	1.9% 59 -
Adults N=15 N_1=32	6.7% 600* -	3.2% 2* -	43.7% 108±270.5 (1–1004)	40.6% 14±19.3 (2–65)	9.3% 60± 7.3 (10–104)	9.3% 6±6.1 (1–13)
Females N=11 N_1=40	15.4% 120±141.4 (20–220)	2.5% 2* -	30% 127±289.4 (1–1004)	40% 19±17.7 (2–53)	5.0% 38±38.6 (10–66)	5.0 % 7±8.5 (1–13)
Males N=18 N_1=43	38.9% 1875±1648.1 (180–4200)	-	34.8% 15±29.6 (1–118)	39.5% 24±24.4 (1–65)	7.0% 78±53.3 (17–114)	4.6% 32±38.2 (5–59)
Total N=31 $N_1$1=83	29.% 1485±1624.5 (20–4200)	1.2% 2* -	32.5% 65±197.9 (1–1004)	39.8% 21±21.3 (1–65)	6.0% 62±48.0 (10–114)	4.8% 19±26.8 (1–59)
		Specimens male to female ratio				
		-	1 : 1.19	1 : 1.38	1 : 1.61	1 : 3.1

[1]based on coproscopy, the intensity of *Eimeria* infection is given in OPG, * only one hare infected, no mean ±SD calculated.

With regard to nematodes in the small and large intestines of the hares, the presence of the following species was noticed: *Trichostrongylus retortaeformis*, *Strongyloides papillosus*, *Trichuris leporis* and *Passalurus ambiguus*. Nematodes *Protostrongylus pulmonalis* were also found in lungs, while no flukes and tapeworms were noticed in hare livers or intestines. The prevalence of nematode infection was higher among adult hares (*S. papillosus* – 3.2%, *T. retortaeformis* – 43.7%, *T. leporis* – 40.6%, *P. ambiguus* – 9.3%, *Protostrongylus pulmonalis* – 9.3%) than in juveniles (*T. retortaeformis* – 25.5%, *T. leporis* – 39.2%, *P. ambiguus* – 3.6%, *Protostrongylus pulmonalis* – 1.9%) (Table 1). The prevalence of infection with those parasites was similar in females and males (Table 1).

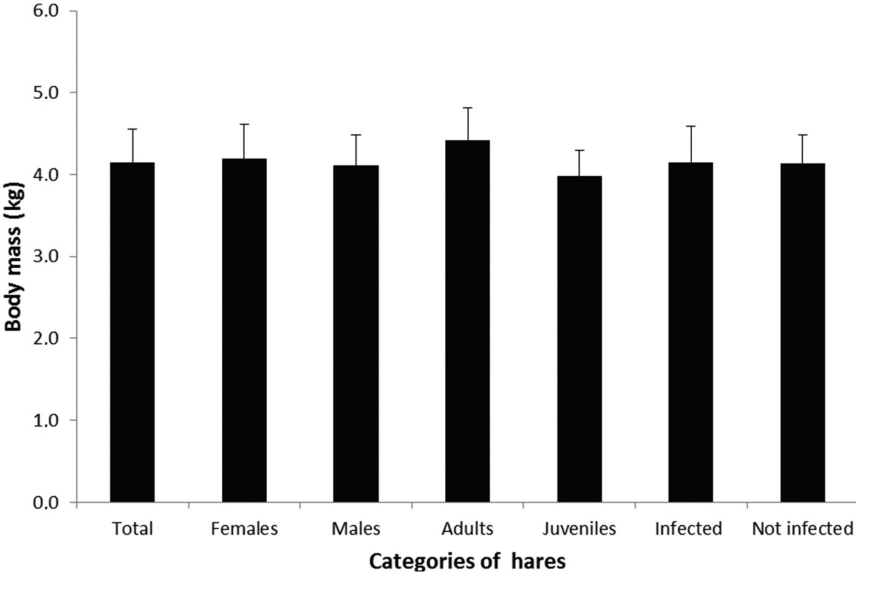

Figure 1. Mean body mass ± SD associated with different hare categories from southern Poland (total number of hares n=83)

Table 2. Results of GLM model on body mass (kg) of hares; sex, age and presence/absence of infection by nematodes were grouping factors. DF – degrees of freedom, MS – mean square, F – ANOVA value for factorial design, P – level of significance

Source of variation	DF	MS	F	P
Infection by nematodes	1	0.036	0.298	0.586
Sex	1	0.075	0.615	0.435
Age	1	2.958	24.225	0.000005
Error	75	0.122		
Infected with nematodes × sex interaction	1	0.006	0.051	0.822
Infected with nematodes × age interaction	1	0.024	0.198	0.658
Sex × age interaction	1	0.027	0.227	0.635
Infected with nematodes × age interaction × age × sex interaction	1	0.006	0.053	0.819

The frequencies of hares infected vs. not infected by nematodes did not differ between sex (χ^2=1.89, P=0.168) and age (χ^2=0.0007, P=0.97). The mean body mass of all hares was 4.15 kg±0.40 kg (Figure 1). GLM model conducted for body mass of hares in which age, sex and presence/absence of nematodes infection were factors showed that there were significant differences between juvenile and adult hares (F=24.225, P=0.000005) (Table 2).

Discussion

To date, only a few studies have been conducted on the parasitic fauna of hares in Poland (Wieczorkowski, 1968; Kozakiewicz et al., 1982; Gundłach et al., 2004; Pilarczyk et al., 2008). In addition Kozakiewicz et al. (1982) and Sadzikowski et al. (2007, 2009) found a high level of infection among hares from semi-closed breeding systems.

The mean *Eimeria* oocysts output found in the research was relatively small (1485±1624.5) (Table 1). Similar results were shown by Aoutil et al. (2005) in France. However, in Poland, Pilarczyk et al. (2008) showed higher infection level with these protozoans (mean 3613 OPG), especially among juvenile hares (mean 68185 OPG). Coccidiosis is particularly dangerous to leverets in which it can be fatal (Alzaga, 2009 after Thulin et al., 2012). In our study the proportion of infected juvenile hares was significantly higher than in adult individuals. All juvenile hares in the study presented by Pilarczyk et al. (2008) were infected with this protozoan and six *Eimeria* species have been found: *E. leporis*, *E. europaea*, *E. robertsoni*, *E. semisculpta*, *E. townsendi* and *E. hungarica*. *E. leporis* and *E. europaea* appear to be dominant species. Strong infection with *Eimeria* species, including the pathogenic *E. leporis*, may be the cause of many hare deaths and, simultaneously, be the vital factor for breeding selection during the first year of the hares' life, especially for those born in late litters (Dziedzic et al., 2000; McCulloch et al., 2004). High infection with coccidia causes loss of appetite and digestion disorders, which often result in intensive diarrhoea. In comparison, in the Czech Republic, the following coccidian species were found: *Eimeria babatica*, *E. europaea*, *E. hungarica*, *E. leporis*, *E. macrosculpta*, *E. robertsoni*, *E. sculpta*, *E. semisculpta* and *E. townsendi* (Pakandl, 1990). In Slovakian studies, the prevalence of eimeriid coccidia reached 91.9% (Dubinský et al., 2010).

Intestinal nematodes were represented by 4 species: *Trichostrongylus retortaeformis*, *Trichuris leporis*, *Passalurus ambiguous* and *Strongyloides papillosus* (Table 1). Slightly greater prevalence of hare infection was noted by Wieczorkowski (1968), Kozakiewicz et al. (1982) and Gundłach et al. (2004) than in the present study (Table 3). *Graphidium strigosum* was not detected in our research, contrary to the studies carried out by Kozakiewicz et al. (1982), Gundłach et al. (2004) and Pilarczyk et al. (2008). The presence of this parasite in hares depends on the occurrence of wild rabbit *Oryctolagus cuniculus* (Anderson, 2000) which has not been reported in this region of Poland.

Symptoms related to the infection with nematodes depend on their location in the host organism and the species of nematode. The occurrence of *Strongyloides papillosus* and *Trichostrongylus retortaeformis* in the small intestine, and *Trichuris leporis* and *Passalurus ambiguus* in the large intestine leads to anaemia, digestive system disorders, and general decrease of wellness (Stefański, 1963; Gundłach and Sadzikowski, 2004). Infection with *Strongyloides papillosus* larvae as well as their migration inflicts damage to internal organs. On the other hand, *Trichostrongylus retortaeformis* and *Trichuris leporis* larvae molting in the intestine's mucus causes significant changes inside the intestine.

Table 3. Prevalence of endoparasites of hare (*Lepus europaeus*) in Poland in post-mortem examinations (Wieczorkowski, 1968; Kozakiewicz et al., 1982; Gundłach et al., 2004)

Parasite	Author			Present study
	Wieczorkowski (1968)	Kozakiewicz et al. (1982)	Gundłach et al. (2004)	
Eimeria sp.	67.0	88.3	55.9	29.0
Fasciola hepatica	1	0	0	0
Cittotaenia denticulata	0	0.6	-	0
Andrya rhopalocephala	2	0.6	-	0
Mosgovoyia pectinata	5	0	-	0
Anoplocephalidae	-	-	0.9	-
Cysticercus pisiformis	1	1.5	10.3	0
Graphidium strigosum	0	44.9	7.6	0
Trichostrongylus retortaeformis	68	65.6	52.8	32.5
Strongyloides papillosus	0	17	0	1.2
Passalurus ambiguus	9	6.3	13.2	6.0
Trichocephalus leporis	37	7.7	47.2	39.8
Protostrongylus pulmonalis	11	0	0	4.8
Number of animals examined	87	506	109	83

The lungworm species, *Protostrongylus pulmonalis*, was found in only 4 out of 83 examined hares in the present study. This lungworm occurred rarely in previous studies conducted in Poland (Czarnowski and Witkowski, 1954; Wieczorkowski, 1968). Only studies performed by authors from Finland demonstrate frequent occurrence of this species of parasite among hares (Laakkonen et al., 2006; Soveri and Valtonen, 1983). Infections of *P. pulmonalis* may cause chronic bronchitis, which in turn results in the general decrease of wellness. Pajerský et al. (1992) and Laakkonen et al. (2006) expose their high pathogenic capabilities.

Many studies reveal conflicting data concerning the parasite load, and composition of parasite species among hares due to numerous factors, including endogenic and environmental ones (Bordes et al., 2007). The level of parasitic infection of hares requires further study, especially now, when there is a visible decline in the number of these animals in Poland. The decrease in the population of hares has been observed for many years in the majority of hunting regions in our country (Kamieniarz and Panek, 2008). By comparing the number of hunted animals in the 1991/1992 and 2007/2008 hunting seasons, hare population decline was the least in the southern areas of Poland, especially in the regions of Kraków and Tarnów (Kamieniarz and Panek, 2008). This tendency can be justified by favourable relief of the land, extensive farming in these regions, and, as observed in this study, low infection rate of hares with endoparasites.

In our study the intensity of nematodes infection was low in comparison to findings presented by other authors (Table 3). The identified parasites might not affect the overall condition of the examined hares; in particular we did not prove significant differences in body mass between infected and non-infected hares by nematodes.

However, any changes in leveret mortality rates might influence the whole population number (Smith et al., 2005; Wibbelt and Frölich, 2005). High infection of juvenile hares suggests the possible threat to the whole population. Thus, long-term studies on parasitic infection of hares should be continued in order to show tendencies among the populations associated with other factors, such as weather conditions and habitat use.

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