

## Parasite load of European brown hares in Austria and the Czech Republic

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**ABSTRACT:** The parasite load of brown hares (*Lepus europaeus*) is of great interest to hunting ground managers and veterinarians. We compared the prevalence and intensity of parasitic infections in 362 hares from Austria and the Czech Republic with respect to age and body weight. Samples of the entire gastrointestinal tract, liver and lungs were collected during autumn hunting events in 2007. The parasite spectrum of hares included *Protostrongylus pulmonalis*, *Graphidium strigosum*, *Trichostrongylus retortaeformis*, *Trichuris leporis*, *Eimeria* spp. and tapeworms. The most prevalent gastrointestinal nematode was *Trichostrongylus retortaeformis*, while only individual specimens of tapeworms such as *Andrya rhopalocephala*, *Mosgovoyia pectinata*, *Cittotaenia denticulata* and *Ctenotaenia ctenoides* were found in subadult hares. A single hare was infected with *Cysticercus pisiformis* in Austria. Lungworms *Protostrongylus pulmonalis* and findings of pneumonia were significantly less prevalent in subadult than adult hares ( $P < 0.01$ ) from both countries and were much less prevalent overall in the Czech Republic ( $P < 0.01$ ). *Graphidium strigosum*, *Trichostrongylus retortaeformis*, *Eimeria* spp. and enteritis were more prevalent in subadult hares. The nematode *Trichuris leporis*, on the other hand, prevailed in adults. The body weight of adult hares was negatively correlated with the intensity of infection by *Protostrongylus pulmonalis* ( $r = -0.67$ ) and *Trichostrongylus retortaeformis* ( $r = -0.73$ ) and the parasite loads served as significant weight predictors in multiple regression equations. This study revealed that parasitic infections of the lungs and intestines influences the health and decreases the body weight of hares in Austrian and Czech hunting grounds.

**Keywords:** *Lepus europaeus*; gastrointestinal parasites; lungworms; coccidia; body weight

The brown hare is one of the most important small game species native to Europe (Pikula et al. 2007), inhabiting all types of hunting grounds from lowland alluvial forests and mixed habitats of hilly country to mountain areas (Pikula et al. 2004). In an analysis of the mortality of 2269 wild brown hares in the Czech Republic, it was found that dietary, parasitic, infectious, toxic, and traumatic factors were responsible for 25%, 25%, 30%, 10%, and 10% of deaths, respectively (Sterba 1982). Brown hares host a broad spectrum of parasites that are of great interest to both hunting ground managers and veterinarians and they are considered important sources of zoonotic agents (Tremel et al. 2007; Bandouchova et al. 2011).

Coccidia are frequent parasites of brown hares. The first comprehensive study on the species spec-

trum of coccidia in hares in Austria was published by Kutzer and Frey (1976). These authors identified a total of eight coccidial species of which *Eimeria robertsoni*, *E. semisculpta* and *E. leporis* were the most common. The species spectra and seasonal dynamics of coccidial shedding were analysed by Chroust (1979, 1984) who also reported eight species, of which *E. robertsoni* and *E. leporis* caused infections in more than 70% of cases in the Czech Republic. Coccidial shedding was shown to be at its peak in the intervals of August to October and March to April. According to Sterba (1982), coccidia accounted for approximately 62% of the total parasitic infections responsible for mortality in brown hares in the Czech Republic. A spontaneous and fatal jejunal intussusception in a brown hare associated with *Eimeria leporis* infection in

Austria has also been reported (McCulloch et al. 2004). Findings of coccidia in the Czech Republic and Austria (both the species spectrum and intensity of infection) are consistent with data published for other neighbouring countries (Czaplinska et al. 1965; Gottschalk 1973; Pellerdy et al. 1974; Barth and Brull 1975; Haupt and Hartung 1977; Nickel and Gottwald 1979; Soveri and Valtonen 1983; Alzaga et al. 2009).

Lungworms are another group of important parasites in the brown hare. The prevalence of *Protostrongylus pulmonalis* (synonymous with *P. commutatus*) was 26.3 and 30.8% in Austria and the Czech Republic, respectively (Kutzer and Frey 1976; Pav 1976). Much wider ranges of 1.1 to 60% were reported from other European countries (Babos 1962; Czaplinska et al. 1965; Grafner et al. 1967; Gottschalk 1973; Nickel and Gottwald 1979). A high prevalence of *P. pulmonalis* was found in adult mountain hares (*Lepus timidus*) in Finland (Soveri and Valtonen 1983; Laakkonen et al. 2006) as well as in their alpine population in Italy (Battisti et al. 2000).

The small-intestinal nematode *Trichostrongylus retortaeformis* is the most common gastrointestinal parasite of brown hares. The prevalence of this parasite was reported to be 85% in Austria (Kutzer and Frey 1976) and 62 and 86% in the Czech Republic (Novak et al. 1966; Pav 1978), with one to 1470 parasite specimens present in hosts. Reports from other European countries as well as from another species, *Lepus timidus*, were within the same range (Irvin 1970; Soveri and Valtonen 1983; Newey et al. 2005; Alzaga et al. 2009). Contrary to most of the above-mentioned studies, Pav et al. (1981) and Grafner (1986) reported a relatively high prevalence and intensity of infection by the gastric nematode *Graphidium strigosum* (25–42 and 44.9%, respectively). Likewise, Sebek (1969) and Irvin (1970) considered this parasite species of great pathologic importance. According to the above authors, *Trichuris* spp. was relatively highly prevalent in Austria, the Czech Republic and most neighbouring countries.

Findings of *Passalurus ambiguus*, a parasite of wild rabbits, are rarely reported, with a prevalence of less than 1%. The prevalence of the hepatic nematode *Capillaria (Hepaticola) hepatica*, occurring in many free-living small mammals, was reported to be 0.5% in Austria (Kutzer and Frey 1976) and was only occasionally found in the Czech Republic (Zajicek 1958).

European brown hares may be infected with the lancet fluke *Dicrocoelium dendriticum* in ar-

reas with sheep at pasture (Graber and Lebrette, 1971; Gottschalk, 1973; Yanchew, 1973; Soveri and Valtonen, 1983; Alzaga et al. 2009). Infection by the liver fluke *Fasciola hepatica* was reported by Kutzer and Frey (1976), Nickel and Gottwald (1979) and Tropilo (1964).

Intermediate stages of tapeworms such as cestodal cysts of *Cysticercus pisiformis* and *Coenurus serialis* are only rarely reported. Some tapeworm species, including *Andrya rhopalocephala*, *A. cuniculi*, *Mosgovoyia pectinata*, *M. ctenoides* and *Cittotaenia denticulata* in particular, may be found in the small intestines of hares (Czaplinska et al. 1965; Kutzer and Frey 1976; Pav et al. 1981; Grafner, 1986).

Detailed knowledge of the parasitofauna of game animals from specific geographic regions may be important in terms of protection against the introduction of new parasites via transfer of animals for repopulation purposes (Bordes et al. 2007). As shown in the above review, there are little current data on the parasite load in hares in Europe. It was therefore, the aim of the present study, to compare the prevalence and intensity of parasitic infections in the brown hare in hunting grounds in Austria and the Czech Republic. Differences in the parasite load were also evaluated with respect to age and body weight.

## MATERIAL AND METHODS

### Hunting bag and habitat description

A total of 225 and 137 brown hares from Austria and the Czech Republic, respectively, were examined in 2007 using autopsy and coprology to determine their parasite load. Samples of the entire gastrointestinal tract, liver and lungs were collected during autumn hunting events from nine Austrian hunting grounds and six hunting grounds from the Czech Republic. The elevation above sea level of the Austrian hunting grounds ranged from 199 to 720 m, while their total area varied from 743 to 4908 ha, with about 35.7% arable land, 23.9% forests, 34.9% meadows and the rest of the land being occupied by water and other habitats. The hunting grounds in the Czech Republic were at elevations above sea level of 170 to 535 m and their total area ranged from 1238 to 1735 ha. The Czech areas included 81.3% arable land, 12.4% forests and only 6.3% meadows.

### Body weight and age determination

Brown hares shot in October to December were sampled to evaluate the species spectra of parasites, and the prevalence and intensity of parasitic infections. Body weight was determined using Pesola 5.0 kg spring scales (Pesola AG, Switzerland) shortly after the animals were shot. Gender was also recorded. The animals were classified as juveniles and adults, according to the Stroh sign, i.e., clinical assessment of distal ulnar epiphyseal closure at 7–8 months of age (Stroh 1931). Age was also checked using another method, i.e., dry eye lens weight measurement (Suchentrunk et al. 1991). The latter method is reliable throughout the hunting season. The eyeball was extracted from the dead hares and fixed in 4% formalin solution for 14 days. Then the lens was dissected from the eyeball, placed on an hourglass and dried at 110 °C for 24 hours. The dry lens was then weighed to an accuracy of 0.001 g. The animals were classified as juveniles born in the same year as they were shot, or adults. The threshold dividing juveniles and adults was represented by the lens weight of 0.280 to 0.290 g.

### Examination for parasites

In the laboratory, samples of the stomach, intestines, liver and lungs were dissected in order to search for gastric and intestinal nematodes and lungworms, and to enable coprological examination for coccidia. Studies were performed on a quantitative and qualitative basis.

Dissection of lungs involved gross examination for surface pathological changes in the lungs and the extent of subpleural lesions, and palpation of the parenchyma. After opening the airways using scissors to cut the trachea and bronchi, lungworms were isolated and preserved in a fixative. Light microscopy was employed to examine fresh scrapings from bronchi and compression mounts of cut lung lesions.

The contents of the stomach and small intestine were separated and placed on a 125 µm sieve for rinsing using a shower. They were then placed in a Petri dish and inspected over a dark background to isolate *Graphidium strigosum* nematodes. The contents of the small intestine were thoroughly mixed with 500 ml of water. Five samples of 10 ml were collected and inspected in a Petri dish using a

binocular microscope. The number of *T. retortaeformis* nematodes isolated was then recalculated for the total volume. Scoleces and tapeworm segments were also isolated from the intestinal contents. The contents of the large intestine and caecum were processed in the same way but a 500 µm sieve was used for preparing samples for isolation of *Trichuris* nematodes. The terminal part of the large intestine containing formed droppings was inspected for the oxyurid nematode *Passalurus ambiguus*. Isolated specimens of nematodes and tapeworms were fixed using 4% formalin and 70% alcohol, respectively.

Fresh faeces were collected from the anus of all hares. Quantitative assessment of coccidia (oocysts per gram of faeces, OPG) was performed using samples of 3 g and a modified quantitative method with a saturated solution of NaCl and a McMaster counting chamber. Coccidial species were identified from samples kept in a 2.5% solution of potassium dichromate after flotation with Sheather's solution, based on size and morphological characteristics (Chroust 1979).

### Statistical analysis

Statistical analyses were performed using Statistica for Windows® 7.0 (StatSoft, Tulsa, OK, USA). Two-sided significance tests for the difference between two proportions (i.e., the prevalence of parasitic infections in males and females, adult and subadult animals, and groups of hares from the Czech Republic and Austria) were employed. The relationship between the intensities of parasitic infections as predictor variables and body weight as a dependent variable was evaluated using Pearson correlation and the results were expressed as correlation coefficients and regression equations.

### RESULTS

The parasite species found in brown hares in Austria and the Czech Republic included *Protostrongylus pulmonalis*, *Graphidium strigosum*, *Trichostrongylus retortaeformis*, *Trichuris leporis*, *Eimeria* spp. and tapeworms. Table 1 demonstrates the differences in prevalence of parasitic infections between subadults and adults as well as total data from both countries. As shown, the most prevalent gastrointestinal nematode was *Trichostrongylus retortaeformis*, while only individual specimens

Table 1. Prevalence of parasitic infections in brown hares from Austria and the Czech Republic

	Prevalence (%)					
	Austria			Czech Republic		
	subadults	adults	total	subadults	adults	total
Examined ( <i>n</i> )	96	85	225	66	65	137
<i>Protostrongylus pulmonalis</i>	16.7	51.7**	37.3	4.5	33.8**	18.2**
<i>Graphidium strigosum</i>	25.0	1.2**	12.4	18.2	1.5**	9.4
<i>Trichostrongylus retortaeformis</i>	91.7	74.1**	82.7	87.9	<b>70.8*</b>	83.2
<i>Trichuris leporis</i>	21.9	56.5**	39.1	7.6	41.5**	31.4
Tapeworms	10.4	0	4.4	6.1	0	2.9
<i>Eimeria</i> spp.	97.9	60**	80.4	95.5	64.6**	79.6
Infected total	97.9	85.9	92.9	93.9	80.1	89.1

\* $P < 0.05$ ; \*\* $P < 0.01$

of tapeworms such as *Andrya rhopalocephala*, *Mosgovoyia pectinata*, *Cittotaenia denticulata* and *Ctenotaenia ctenoides* were found in subadult hares. There was also a single case of a hare infected with *Cysticercus pisiformis* in Austria. Lungworms *Protostrongylus pulmonalis* as well as findings of pneumonia were significantly less prevalent in subadult than adult hares ( $P < 0.01$ ) from both countries, with a particularly low prevalence in the Czech Republic ( $P < 0.01$ ). *Graphidium strigosum*, *Trichostrongylus retortaeformis* and *Eimeria* spp., as well as findings of enteritis, were more prevalent in subadult hares. However, the nematode *Trichuris leporis* prevailed in adults. The intensity of parasitic infections is shown in Table 2. There were considerable differences in the maximum and mean OPG numbers in subadult hares from Austria and the Czech Republic. The maximum *Trichostrongylus*

*retortaeformis* intensity in subadult hares from Austria significantly exceeded levels from the Czech Republic, where lower loads of *Protostrongylus pulmonalis* were also found. The body weight of adult hares was negatively correlated with the intensity of infection with *Protostrongylus pulmonalis* ( $r = -0.67$ , Figure 1) and *Trichostrongylus retortaeformis* ( $r = -0.73$ , Figure 2). Multiple regression resulted in an equation for body weight of  $4.35 - 0.21 * Protostrongylus - 0.48 * Trichostrongylus - 0.12 * Trichuris - 0.08 * Eimeria$  ( $r = 0.77$ , standard error = 0.27,  $n = 134$ ,  $P < 0.01$ ), where the first two parasite intensities were significant predictors, but the importance of *Trichuris* and *Eimeria* infections was negligible. The intensity of *Trichostrongylus retortaeformis* infection was positively correlated with that of *Eimeria* spp. ( $r = 0.78$ ) and *Protostrongylus pulmonalis* ( $r = 0.74$ , Figure 3).

Table 2. Intensity of parasitic infections in brown hares from Austria and the Czech Republic

	Ranges of parasite numbers			
	Austria		Czech Republic	
	subadults	adults	subadults	adults
Examined ( <i>n</i> )	96	85	66	65
<i>Protostrongylus pulmonalis</i>	4–28 ( $\bar{x} = 11.9$ )	2–24 ( $\bar{x} = 14.1$ )	3–18 ( $\bar{x} = 8.3$ )	3–22 ( $\bar{x} = 10.7$ )
<i>Graphidium strigosum</i>	4–12 ( $\bar{x} = 8.6$ )	8 ( $\bar{x} = 8$ )	4–11 ( $\bar{x} = 6.2$ )	14 ( $\bar{x} = 14$ )
<i>Trichostrongylus retortaeformis</i>	44–1240 ( $\bar{x} = 358.7$ )	36–348 ( $\bar{x} = 148.0$ )	62–780 ( $\bar{x} = 342.0$ )	44–484 ( $\bar{x} = 160.0$ )
<i>Trichuris leporis</i>	3–24 ( $\bar{x} = 8.7$ )	4–12 ( $\bar{x} = 10.6$ )	4–12 ( $\bar{x} = 7.1$ )	4–18 ( $\bar{x} = 9.6$ )
<i>Eimeria</i> spp.	800–1 680 000 ( $\bar{x} = 47416$ )	200–38 800 ( $\bar{x} = 5214$ )	800–46 800 ( $\bar{x} = 14168$ )	200–33 400 ( $\bar{x} = 3762$ )

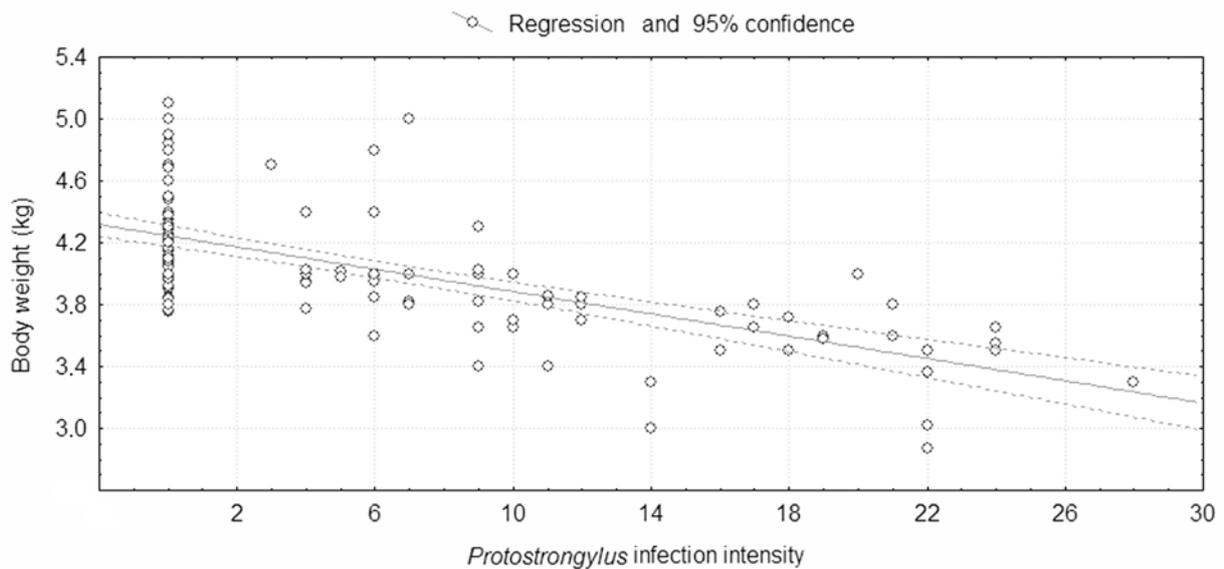


Figure 1. Regression between the body weight of adult hares and the intensity of infection by *Protostrongylus pulmonalis* ( $r = -0.67$ ). Body weight of hares =  $4.24 - 0.036 * \text{Protostrongylus infection intensity}$

**DISCUSSION**

Comparison of the present results on *P. pulmonalis* with earlier reports by Kutzer and Frey (1976) and Pav (1976) from Austria (26.3%) and the Czech Republic (30.9%), respectively, indicates that the parasite is more prevalent in Austrian hares than in Czech hares, both in juveniles and adults, the

latter being more frequently affected by pneumonia. These observations, as well as the lower percentage of parasite-free hares in Austria, can be explained by the occurrence of habitats favourable for intermediate hosts such as snails and therefore a higher risk of lungworm infection in this country. Austrian hunting grounds are more often composed of meadows and other natural vegetation

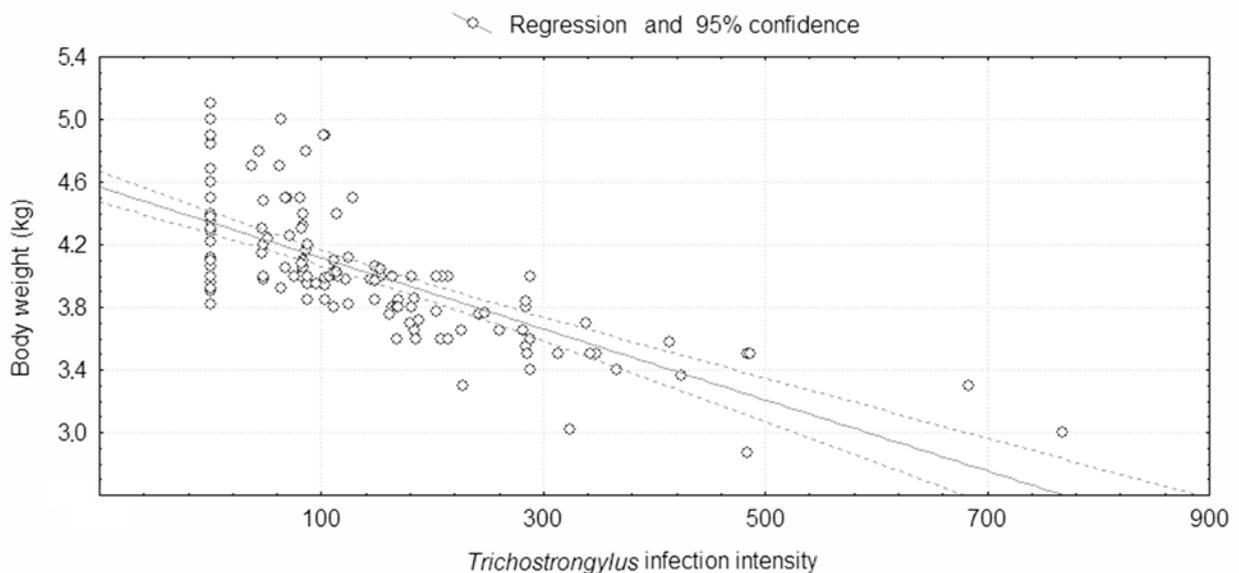


Figure 2. Regression between the body weight of adult hares and the intensity of infection by *Trichostrongylus retortaeformis* ( $r = -0.73$ ). Body weight of hares =  $4.34 - 0.0023 * \text{Trichostrongylus infection intensity}$

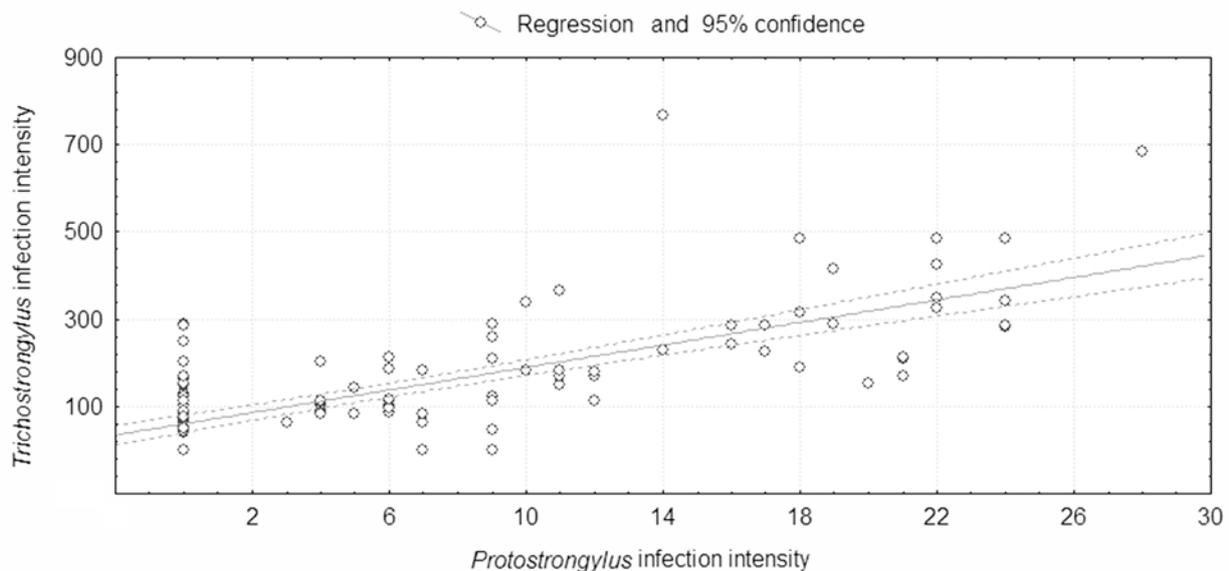


Figure 3. Regression between the intensity of infection by *Trichostrongylus retortaeformis* and *Protostrongylus pulmonalis* ( $r = 0.74$ ).  $Trichostrongylus$  infection intensity =  $60.26 + 12.90 * Protostrongylus$  infection intensity

(34.2%) and thus include diverse communities of herbs. On the other hand, large-scale agricultural production units with a high percentage of arable land and monocultures (81.3%) still prevail in the Czech Republic where meadows and other habitats represent only 6.3% of the area.

Adverse effects of *P. pulmonalis* on the health, body condition and weight of hares were reported by Kutzer and Frey (1976). According to these authors, hares affected by this lungworm die twice as frequently due to bacterial infections than non-parasitised hares. The marked pathogenicity of lungworms in mountain hares (*Lepus timidus*) was also found by Battisti et al. (2000) in Italy. Interestingly, Laakkonen et al. (2006) reported that the condition and weight of Finnish hares did not show any significant association with the intensity of lungworm infection and Frolich et al. (2003) found all lung samples from 116 German hares negative for lungworms. Our findings in the most serious parasitic infections of older hares in particular, included extensive subpleural lesions comprised of 20 to 50% of the lung surface, which was characterized by lung tissue atelectasis and interstitial pneumonia with increased septal thickness. After dissecting the atelectatic lungs, it was possible to collect and observe numerous larvae and eggs of lungworms using scraping and compression procedures. The abundant bronchial mucus also contained numerous larvae. These findings are

consistent with typical parasitic pneumonia cases and result in favourable conditions that promote bacterial or viral infections. The general condition and state of health of hares subsequently deteriorates as witnessed by the fact that these cases were found mostly in hunting grounds characterised by the lowest number of hunting bags and the highest mortality, and in hares of lower body weight.

The present study is also consistent with the previously published papers of Kutzer and Frey (1976) and Sterba (1982) demonstrating the importance of coccidia in the mortality of hares. Important coccidian species include *E. robertsoni* and *E. leporis*, found in 87 and 62%, respectively, of coccidian-positive hares. Both species occur with a high intensity of infection. Severe infections, mostly of juvenile hares, result in enteritis of large segments of the small intestine and soft or even diarrhoeal contents of the large bowel and rectum. In some of these hares there were mucosal nodules in the jejunum and ileum as well as yellow-whitish layers formed by the developing coccidial stadia and suppurative or necrotic lesions. The clinical importance of *E. leporis* was also documented by McCulloch et al. (2004), who reported this coccidian species to be the primary cause of death in hares. Other species of coccidia were found with a lower prevalence and intensity of infection (*E. europaea* 27.3%, *E. septentrionalis* 19.4%, *E. semisculpta* 18.6%, *E. hungarica* 11.2% and *E. townsendii* 8.1%). Interestingly, the

influence of *Eimeria* infections on the body weight of adult hares was negligible in this study.

The nematode *T. retortaeformis* was found with a higher prevalence and intensity of infection in juvenile hares. Severe infections resulted in chronic enteritis, mostly in the duodenum and jejunum, characterised by hyperaemia, petechiae and epithelial erosions sometimes extending into deeper layers of the mucosa. The importance of this nematode as a pathogenic agent inducing mortality or body weight loss was demonstrated by Haupt and Hartung (1977), Gottschalk (1973) and Boch and Schneidawind (1988). Importantly, Newey et al. (2005) reported that *T. retortaeformis* infection results in a strong reduction in the body condition and fertility of females but does not influence their survival. On the other hand, a surprisingly low intensity of infection by *T. retortaeformis* was recently reported in brown hares from Slovak Republic (Dubinsky et al. 2010).

The prevalence and intensity of infection by *T. leporis* in the present study was similar to previously reported results and it may be hypothesised that this parasite does not influence the health status of hares in the same way as *T. retortaeformis*. The nematode *T. sylvilagi*, also reported to occur in European brown hares, was not found in specimens examined in the present study. Likewise, tapeworms were found only in sporadic cases and therefore appear to have only minor importance for the health status of hares.

In conclusion, this study showed that parasitic infections, both of the lungs and intestines, influenced the health status as well as decreased the body weight of hares in Austrian and Czech hunting grounds. Regression analysis revealed that mixed infections combined to enhance the adverse effects of the parasites.

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