

## Endoparasites in European hares (*Lepus europaeus*) under gamekeeping conditions in the Czech Republic

D. LUKEŠOVÁ<sup>1\*</sup>, I. LANGROVÁ<sup>2</sup>, J. VADLEJCH<sup>2</sup>, I. JANKOVSKÁ<sup>2</sup>, J. HLAVA<sup>2</sup>, P. VÁLEK<sup>2</sup>, Z. ČADKOVÁ<sup>2</sup>

<sup>1</sup>Department of Animal Science and Food Processing in the Tropics and Subtropics, Institute of Tropics and Subtropics, Czech University of Life Sciences, 165 21 Prague 6 – Suchbát, Czech Republic,

\*E-mail: [lukesd@its.czu.cz](mailto:lukesd@its.czu.cz); <sup>2</sup>Department of Zoology and Fisheries, Faculty of Agrobiological Sciences, Food and Natural Resources, Czech University of Life Sciences Prague, Kamýcká 957, 165 21 Prague 6 – Suchbát, Czech Republic

### Summary

During December 2007 and November – April 2008/2009, 128 gastrointestinal tracts and 1680 faecal samples of hares from different parts of the Czech Republic were examined. Evaluation of the faeces samples indicated that the samples could have been infected with any one of the following parasites: *Eimeria* spp., *Trichostrongylus retortaeformis*, *Trichuris leporis*, *Graphidium strigosum*, *Strongyloides* sp., *Protostrongylus* spp. and Anoplocephalidae tapeworms. *Trichostrongylus retortaeformis* was the most common nematode (75.8 %). 39.8 % of the animals were infected with *Trichuris leporis*. Only four hares were infected with *Graphidium strigosum*. *Eimeria* spp. was the most commonly occurring parasite (90.5 %). The following species were found: *Eimeria coquelinae*, *E. cabareti*, *E. leporis*, *E. babatica*, *E. europea*, *E. pierrecouderti*, *E. macrosculpta*, *E. orbiculata* and *E. stefanski*. *Eimeria coquelinae* was the most prevalent species (68.4 %). *Eimeria cabareti* and *E. leporis* were the next most frequently found species (64.5 % and 57.8 %, respectively).

Keywords: European hare; Central Europe; endoparasites; prevalence; seasonal dynamics

### Introduction

Reports on endoparasites in hares are sporadic (especially recently), with none whatsoever from the region of Central Europe. Moreover, hares in the Czech Republic live almost exclusively in areas kept by game keeping organizations. These organizations provide the animals with supplementary feed and treat their illnesses in winter. In autumn, certain specimens are permitted to be shot.

Hares in Central Europe are traditional game animals, which usually occur in Europe in abundant numbers (Strauss *et al.*, 2008; Fettingner *et al.*, 2010). Unfortunately their counts in the Czech Republic have been dropping in recent times. There are several causes, but the main rea-

sons are changes in rural landscape and general changes in agriculture. A number of game keeping organizations take very good care of their hares because they are traditionally a very popular game animal. At their suggestion, we investigated current parasitary conditions of these animals.

Many studies of the endoparasites of European hares were conducted in the latter half of the twentieth century (Haupt & Hartung, 1976; Kutzer & Frey, 1976; Nickel & Gottwald, 1979; Forstner & Ilg, 1982; Soveri & Valtonen, 1983; Pakandl, 1990; Allgöwer, 1992; Böckeler *et al.*, 1994). Recently, studies have become rarer i.e., from France by Bordes *et al.* (2007), from Poland by Pilarczyk *et al.* (2008) and from Slovakia by Dubinský *et al.* (2010).

The objectives of this study were as follows: 1) to observe the prevalence and abundance of gastrointestinal (GI) parasites of European hares in game keeping shooting-ground conditions during two winter seasons, 2) to monitor seasonal dynamics of exogenous stage excretion during the winter and spring season. 3) to evaluate the importance of parasites for hares in the Czech Republic.

### Materials and methods

Parasite data came primarily from 128 hares shot dead during December 2007 and December – January 2008/2009, always from 8 localities (game keeper areas). The localities stretched from the lowlands of intensive agriculture (landscape dominated by fields) to the highlands.

The size of the gamekeeping areas ranged from 750 ha to 1860 ha, with forest-covered areas ranging from 1.5 % to 37.4 % (highlands). Altitudes were from 184 to 610 m.

During the slaughtering periods, hunters were asked to collect the GI tract from hares from different parts of the Czech Republic, and send them to the Section of Parasitology, Czech University of Life Sciences Prague. Furthermore, the faecal samples were collected from the same 8

Table 1. Prevalence and intensity of parasitism among hares (*Lepus europaeus*) in the Czech Republic

Parasite	Number infected	Percentage of infection (%)	Intensity	
			Mean ± SD	Range
<i>Trichostrongylus retortaeformis</i>	97	75.8	158.9 ± 377.3	4 – 2691
<i>Trichuris leporis</i>	51	39.8	13.4 ± 12.9	1 – 47
<i>Graphidium strigosum</i>	4	3.9	1.5 ± 0.6	1 – 2

localities (400 samples during December 2007, 160 samples each month from November 2008 to April 2009). The viscera (abomasum, small intestines colon and caecum) of all hares were collected and processed for worm recovery, enumeration and identification by the methods described by Eysker and Kooyman (1993).

The *Eimeria* oocysts were determined using the descriptions of Pellérdy (1956), Bouvier (1967), Levine and Ivens (1972), Pakandl (1990) and Aoutil *et al.* (2005). Statistical analysis of data was conducted by the statistical program Statistica.cz (StatSoft USA, ANOVA, POST-HOC test).

Table 2. Parasite faecal stage excretion in hares (*Lepus europaeus*) in the Czech Republic

Parasite	Number of faecal samples	Individual positive faecal samples (%)	Intensity*	
			Mean ± SD	Range
<i>Eimeria</i> spp.	800	90.5	593.0 ± 881.8	4 – 9226
<i>Trichostrongylus retortaeformis</i>	800	88.2	121.8 ± 380.1	4 – 1045
<i>Graphidium strigosum</i>	800	7.3	11.9 ± 10.6	4 – 38.5
<i>Trichuris leporis</i>	800	19.2	40.5 ± 63.1	4 – 98
<i>Strongyloides</i> spp.	800	6.5	14.3 ± 11.6	4 – 27
<i>Protostrongylus</i> spp.	800	25.7	19.8 ± 75.5	4 – 423.5
Anoplocephalidae	800	0.87	7.5 ± 6.2	4 – 21

\*eggs/oocysts/larvae per gram of faeces

The faecal samples were stored in plastic tubes at 4 °C for a week prior to analysis. All the samples from the hares were examined individually for GI nematode eggs and coccidian oocysts using the modified Breza flotation solution (Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> and MgSO<sub>4</sub> with specific gravity 1.35 g.cm<sup>-3</sup>) and McMaster method (Wetzel, 1951). A Baermann apparatus was used to separate 1<sup>st</sup> stage lungworm larvae from faecal samples. *Cryptosporidium* spp. was detected by means of the Miláček and Vítovec method (1985).

Isolated oocysts, eggs and larvae were investigated using an Olympus BX41 microscope. Oocysts were measured using an integrated computerized system, and photographed with an Olympus SP-350 digital camera mounted on an Olympus BX41 microscope using phase contrast imaging with a 100x objective oil immersion lens.

Eggs, L<sub>1</sub> larva and adult helminth parasites were determined according to Levine (1980) and Kotrlá *et al.* (1984).

## Results

Three species of parasites were identified in the GI tracts of 128 hares examined: *Trichostrongylus retortaeformis*, *Trichuris leporis* and *Graphidium strigosum*. *Passalurus ambiguus*, *Strongyloides* sp. and tapeworms were not found in the intestines of the examined hares.

*T. retortaeformis* was the most common nematode (97 animals positive, 75.8 %), 39.8 % (n = 51) of the animals infected with *Trichuris leporis*. Only four hares were infected with *Graphidium strigosum*. The mean intensity of infection with *T. retortaeformis* was 158.9 (4 – 2691), with *T. leporis* 13.4 (1 – 47) - Table 1.

Significant differences in gender parasite distribution were not detected (p ≥ 0.05). Gastrointestinal parasites in hare excretion are shown in Table 2. Evaluation of the faeces samples indicated that they could have been infected with

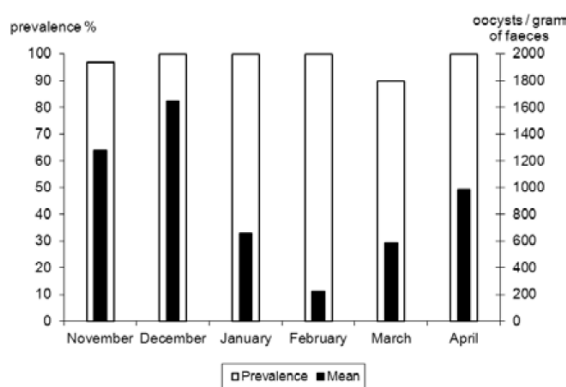


Fig. 1. Seasonal dynamics of exogenous parasitic stage excretion in feces – *Eimeria*

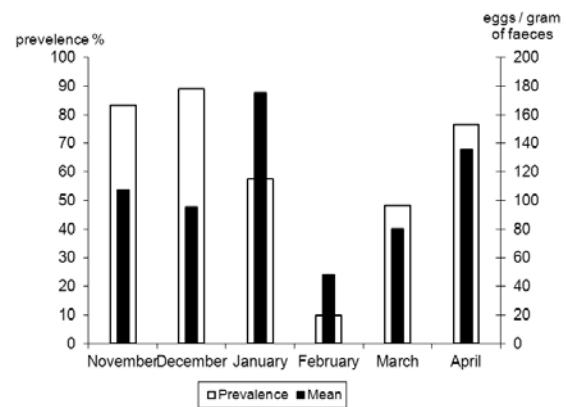


Fig. 2. Seasonal dynamics of exogenous parasitic stage excretion in feces – *Trichostrongylus* sp.

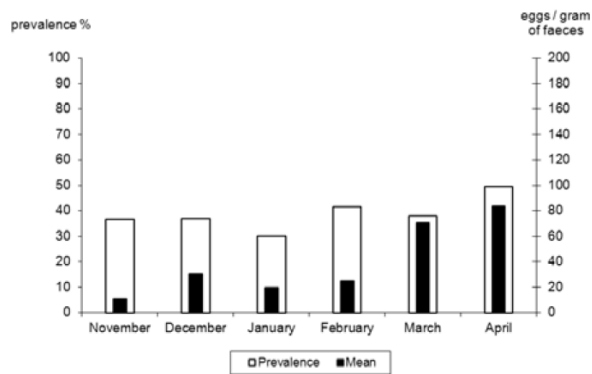


Fig. 3. Seasonal dynamics of exogenous parasitic stage excretion in feces – *Trichuris* sp.

any one of the following parasites: *Eimeria* spp., *Trichostrongylus retortaeformis*, *Trichuris leporis*, *Graphidium strigosum*, *Strongyloides* sp., *Protostrongylus* spp. and Anoplocephalidae tapeworms.

*Eimeria* spp. was the most commonly occurring parasite being recorded from hare faeces (90.5 % faecal samples), followed by *T. retortaeformis* (88.2 %) and *T. leporis* (19.2 %) - Table 2. Anoplocephalidae showed the lowest prevalence and intensity of the exogenous stages – eggs (0.87 %).

Table 3. *Eimeria* faecal oocyst excretion in hares (*Lepus europaeus*) in the Czech Republic

Parasite species	Number of faecal samples	Individual positive faecal samples (%)	Intensity	
			Mean ± SD	Range
<i>Eimeria coquelinae</i>	800	68.4	521.2 ± 823.2	7 – 4 393
<i>Eimeria cabareti</i>	800	64.5	318.7 ± 519.3	18 – 3 948
<i>Eimeria leporis</i>	800	57.8	203.6 ± 380.1	7 – 2 236
<i>Eimeria babatica</i>	800	39.5	47.2 ± 69.6	4 – 287
<i>Eimeria europea</i>	800	34.7	22.8 ± 36.6	7 – 178
<i>Eimeria pierrcouderti</i>	800	15.9	34.4 ± 52.4	4 – 287
<i>Eimeria macrosulpta</i>	800	7.3	10.6 ± 18.3	4 – 123
<i>Eimeria orbiculata</i>	800	8.3	10.1 ± 12.5	4 – 56
<i>Eimeria stefanski</i>	800	1.8	7.4 ± 14.4	4 – 59

Seasonal dynamics of exogenous parasitic stage excretion in feces are given in Figs 1 to 3. A significant decrease in the intensity of the *Eimeria* oocyst excretion was observed in January and, in particular, February ( $660.5 \pm 797.5$ ,  $224.4 \pm 228.1$  respectively). A decline in the prevalence and intensity of egg excretion in February was also observed for *Trichostrongylus retortaeformis* (9.8 %,  $47.9 \pm 110$ ). The presence of *Trichuris* eggs in the feces increased in the spring (in April – 49.5 %, average EPG  $83.5 \pm 49.3$ ). Nine different species of *Eimeria* were identified from 800 faecal samples of hares collected from 8 shooting-grounds in the Czech Republic: *Eimeria coquelinae*, *E. cabareti*, *E. leporis*, *E. babatica*, *E. europea*, *E. pierrcouderti*, *E. macrosulpta*, *E. orbiculata* and *E. stefanski*. The species detection and prevalence rates are given in Table 3. *Eimeria coquelinae*, which occurred in 68.4 % of the samples, was the most prevalent species among the hares. *Eimeria*

*cabareti* and *E. leporis* were the next most frequently found species. They occurred in 64.5 and 57.8 % of the samples, respectively. Other species were found in 39.5 % or less of the samples. *Eimeria stefanski* was the most infrequently occurring species (Table 3).

*Cryptosporidium* spp. was detected in 5 of the 360 examined faecal samples of hares. In the current study, no significant differences in hare parasite infection among all localities were discovered ( $p \geq 0.05$ ).

## Discussion

The hare is an important game animal in the Czech Republic. Generally we take the indigenous population into account. Although in some areas, animals were released that had been previously caught in neighbouring countries (i.e. Hungary). The population of wild rabbits, which could affect parasites in hares, is currently negligible in the Czech Republic.

The parasite species community of the Czech game hare is very limited, only three species of nematodes were recorded in the GI tracts of game hares in the Czech Republic: *Trichostrongylus retortaeformis*, *Trichuris leporis* and *Graphidium strigosum*. Both *Passalurus ambiguus* and tapeworms were not observed.

In the present study, as well as in surrounding countries, *T. retortaeformis* was the most common species. Studies from surrounding European countries showed that infection varies from 75.8 % to 96.8 % (Haupt & Hartung, 1976; Kutzer & Frey, 1976; Forstner & Ilg, 1982; Allgöwer, 1992). A lower prevalence from Finland was reported by Soveri and Valtonen (1983) (54.2 %) and a very low prevalence was reported from Belarus by Shimalov (2001) – 18.2 %. The occurrence of *T. retortaeformis* is largely determined by appropriate environmental conditions in Central Europe. Only when temperatures drop below 10 °C, does exogenous development cease (Crofton, 1946). The second most important nematodes of hares is *T. leporis*. The prevalence from other European countries was found to range between 13.2 % (Belarus – Shimalov, 2001) to 66.7 % (Germany – Haupt & Hartung, 1976). In agreement to the many reports from surrounding European

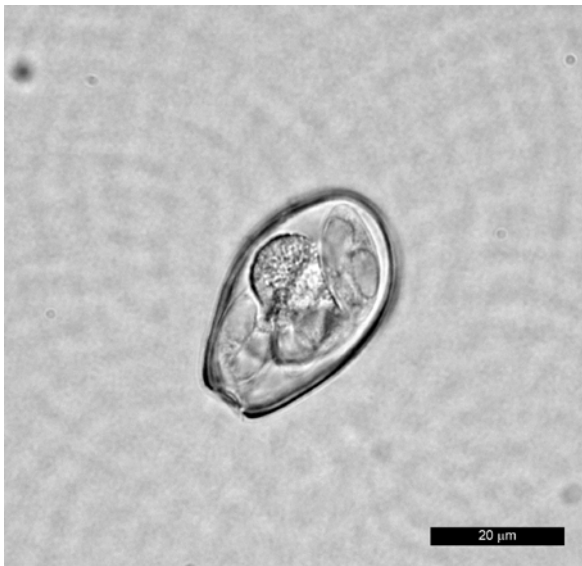


Fig. 4. The photomicrograph of sporulated *Eimeria coquelinae* oocyst

countries (Kutzer & Frey, 1976; Nickel & Gottwald, 1979; Soveri & Valtonen, 1983; Allgöwer, 1992; Shimalov, 2001), *Graphidium strigosum* was rarely found in the present study (3.9 %); this is probably related to the lack of wild rabbits (Broekhuizen & Kemmers, 1976).

Comparison of infection found both in the animals and faecal samples show that the highest rate of discrepancies was observed in *Trichuris leporis*. This can be due to the great number of both *Trichuris* larvae and young specimens found in the intestines of hares in the winter season. A very small number of tapeworms eggs (Anoplocephalidae) were recorded in the faeces (0.87 %), no tapeworms were found in the intestines of the hares.

*Strongyloides* sp. is one of the many tiny parasites that can be easily overlooked. These parasites can only be found using a microscope, as shown through our experience with parasites of sheep (Langrova *et al.*, 2008). However, results from coproscopical examinations show that *Strongy-*

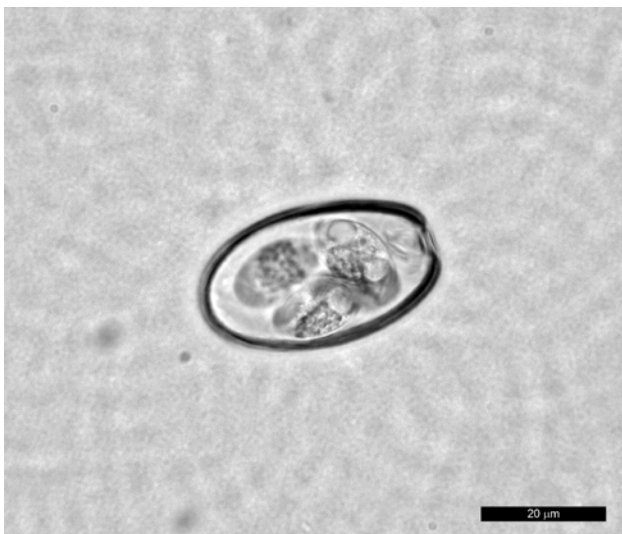


Fig. 5. The photomicrograph of sporulated *Eimeria cabareti* oocyst

*loides* (most likely *S. papillosus*) is a relatively common parasite of lagomorphs.

The dynamics of exogenous parasitic stage excretion were monitored in the second year, from November 2009 to April 2009. One purpose of this investigation was to study the effects of the administration of anticoccidial supplementation in the diet; in December 2009, a one-time application of Robenidin hydrochloride – 66 mg of the preparation per 1 kg of fodder mixture, 0.6 kg food/hare/day – was added to fodder.

As Fig. 1 demonstrates, this drug suppresses the intensity of infection for no more than three months however, it does not affect the prevalence of this parasite.

The paucity of the parasite community could be related to both the low and ever decreasing densities of hares in the Czech Republic, and also due to the excellent care of game hares in the nation's gamekeeper grounds (including veterinary care, winter fodder).

Coccidia are among the most potent pathogenic parasites of hares, and in combination with lung and digestive tract nematodes, they have become a major regulatory factor of the hare population (Chroust, 1984). In previous studies, the prevalence rates of coccidial infections in hares in the Czech Republic were very high, ranging between 92.5 % and 100 % (Ryšavý, 1954; Chroust, 1979, 1984; Jirouš, 1979; Pakandl, 1990) and likewise in hares from surrounding countries such as Germany, Austria or Poland: between 63.3 % and 100 % (Kutzer & Frey, 1976; Haupt & Hartung, 1976; Forstner & Ilg, 1982; Allgöwer, 1992; Böckeler *et al.*, 1994; Pilarczyk *et al.*, 2008). During coprological examination, oocysts of the following species were found: *Eimeria coquelinae*, *E. cabareti*, *E. leporis*, *E. babatica*, *E. europea*, *E. pierrecouderti*, *E. macrosculpta*, *E. orbiculata* and *E. stefanski*. Oocyst differentiation was performed according to Pellérdy (1956), Bouvier (1967), Levine and Ivens (1972), Pakandl (1990) and Aoutil *et al.* (2005). The species were named according to Aoutil *et al.* (2005), however, we determined differences in the shapes of some species. The shapes of *Eimeria coquelinae* as well as *E. cabareti* differed from their depictions in microphotographs and sketches by Aoutil *et al.* (2005). We observed the shape of *Eimeria coquelinae* which was ovoid and not short ellipsoid (according Aoutil *et al.*, 2005) while the shape of *E. cabareti* was ellipsoid, and not ovoid (Figs 4 and 5). Our observation of the shapes of these coccidia species coincide with the microphotographs taken by Pakandl (1990).

This study confirms that coccidiosis is the most significant threat to game hares in the Czech Republic. Other species, especially *Trichostrongylus retortaeformis* and *Trichuris* spp. are a normal part of the parasite fauna of hares. However, we believe that these parasites significantly affect neither the health nor the population of hares.

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