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ORIGINAL PAPER

# *Cephenemyia stimulator* and *Hypoderma diana* infection of roe deer in the Czech Republic over an 8-year period

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**Abstract** A survey of naso-pharyngeal and subcutaneous myiasis affecting roe deer (*Capreolus capreolus*) was conducted in the Czech Republic over an 8-year period (1999–2006). A total of 503 bucks and 264 does from six hunting localities were examined. The sampling area comprised predominantly agricultural lowlands and a mountain range primarily covered by forest. Since 1997, the deer have been treated each winter across the board with ivermectin (150 mg/kg, CERMIX® pulvis, Biopharm, CZ). Parasites found were the larvae of *Hypoderma diana* and *Cephenemyia stimulator*. There were no significant differences in warble fly infection among captured animals in the individual hunting localities. Overall, 146 (28.8 %) of 503 animals (bucks) were infected with *Cephenemyia stimulator* larvae; body size of the second instar larva reached 13–18 mm. The prevalence ranged from 16.1 to 42.9 % per year, and the mean intensity from 6 to 11 larvae per animal. Additionally, a total of 264 roe deer (does) were examined for *H. diana* larvae, and 77 (29.1 %) were found to be positive; body size of the second instar larva reached 17 mm. The prevalence ranged from 18.8 to 50.0 % per year, and the mean intensity from 13 to 22 larvae per animal. The results showed that the bot flies, *Cephenemyia stimulator* as well as *H. diana*, are common parasites in roe deer in the Czech Republic, and that through the help of treatment (ivermectin), it is possible to keep parasite levels low. The body weights of infected and non-infected *H. diana* deer did not differ significantly.

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## Introduction

Bot fly infection is a notorious problem throughout the world. Larvae of *Hypoderma diana* cause subcutaneous myiasis, and the larvae of *Cephenemyia stimulator* are found in the nasal passages and throat pouches of the host. Both specimens of bot fly can be found in all species of wild ruminants. However, they are most common in game roe deer (Červený et al. 2004).

Hunters believe that these bot flies significantly influence the general health status as well as the immune system of the body of infected animals. However, studies concerning definite influences of the bot fly on the host organism are sporadic; with respect to *H. diana*, there is no information in science journals. Unfortunately, reports on the prevalence of bot flies in various countries are rare; however, detailed information regarding nasal bots come from Poland and Hungary (Dudziński 1970a, b; Sugár 1975; 1978; Kiraly and Egri 2004; 2007).

The aims of the study were as follows: (1) determine the prevalence and intensity of nasal and subcutaneous bot fly infections in roe deer in the Czech Republic, (2) describe the seasonal dynamics of infection of *Cephenemyia stimulator* second and third stage larvae and (3) establish differences between the weight of *H. diana* infected and non-infected does.

## Materials and methods

From 1999 to 2006, a total of 767 roe deer (*Capreolus capreolus*) from six hunting localities of the Czech Republic

were examined for the larvae of the warble fly *H. diana* (503 animals) and *Cephenemyia stimulator* larvae (264 animals). The sampling area comprised lowlands with a predominantly agricultural use and a mountain range primarily covered by forest (altitude ranging from 210 to 744 m above sea level). Since 1997, the deer have been treated each winter across the board with ivermectin (150 mg/kg, CERMIX® pulvis, Biopharm, CZ), 1 kg of the preparation per 9 kg of a feed mixture, 0.5 kg/10 kg of the body weight per day. The larvae of *Cephenemyia stimulator* were collected from 503 animals during buck hunting season (May 16–September 30). The heads of the animals were immersed in water for 24 h, and examinations were carried out through rinsing of the nasal cavities, sedimentation of the rinses and their quantitative evaluation for the presence of bot flies in the larval stage. Larvae were fixed in 75 % ethanol. To ensure result accuracy, only the second and third stage larvae were used. Identification of the species and larval stages was carried out according to Grunin (1957), Chvála (1980) and Papp and Szappanos (1992). Briefly, body size of the second instar larva reaches 13–18 mm. Dorsal body side is almost without any spines. Body segment 10 is bare. Larvae of the third instar were identified according to outgrowths of pseudocephalon (meeting each other), and spines on the dorsal side of the body are distinctly larger than those on the ventral body side and dark in mature larvae.

A total of 264 does were investigated for the larvae of *H. diana*. The larvae were collected between November 1 and December 31. The parasites were discovered during skinning, on the backs and sides of the animals. Larvae were removed from the hides, counted and preserved in 75 % ethanol. Classification of larval stages as well as identification of *H. diana* were conducted according to Grunin (1962), Chvála (1980) and Papp and Szappanos (1992). Briefly, body size of the second instar larva reaches 17 mm. Front margins of the dorsal side of the thoracic segments 1–2 are usually armed with belts of spines. The ventral side have belts of spines usually developed from segments 2 to 5, missing on segments 3 to 4. Belts of spines on both the front and rear margins of segments are arranged in 10 irregular rows. Larvae of the third instar were identified primarily according to the shape and location of the peritreme. The all hunted deer were weighed (the weights given are per animal without its intestines, head and legs). The legs were severed at the forearm and hock joint, respectively.

Basic descriptive statistics (mean, minimum and maximum and standard deviation) were computed for number of parasites (Tables 1 and 2) and for body weight of roe deer (Table 3). Linear regression of body weight as independent variable and number of parasites as dependent variable was computed. One-way ANOVA was used to evaluate if there is a difference in the number of parasites in hosts among localities. Statistical analysis was carried out with the computer software Statistica ver. 9 (StatSoft 2009).

## Results

In the present study, a total of 503 bucks and 264 does *Capreolus capreolus* from six hunting localities of the Czech Republic were examined for the larvae of the warble fly *H. diana* or *Cephenemyia stimulator* larvae. There were no significant differences in the warble fly infection among captured animals in the individual hunting localities.

From 1999 to 2006, a total of 503 roe deer were examined for the larvae of *Cephenemyia stimulator*. A total of 146 animals were infected with nasal bot; over an 8-year period, the mean prevalence ranged from 16.1 to 42.9 % and the mean intensity of infection was from 6.2 to 11.2 larvae per animal. Results from each year are shown in Table 1.

During the hunting season, a decreasing number of *Cephenemyia stimulator* larvae were recorded. The mean intensity of infection was highest in May and gradually decreased until September (Table 1).

During the observation period, a total of 264 roe deer were examined for the larvae (only second instars) of the warble fly *H. diana*. Of those, 77 were tested positive. Over an 8-year period, the mean prevalence ranged from 18.8 to 50.0 % and the mean intensity of infection from 12.8 to 21.8 larvae. The prevalence and mean intensity rates in animals for each year are reported in Table 2. The regression between the number of *H. diana* larvae and body weight was found as very poor ($R$=0.08) (Fig. 1).

## Discussion

Parasite infection is a serious health problem not only in small ruminants such as sheep (Langrová et al. 2008; Makovcová et al. 2008, 2009; Morgan and Van Dijk 2012; Taylor 2012) but also in wild ruminants (Schwarz et al. 2011; Bolukbas et al. 2012). Most studies in this field have focused on helminths in roe deer (Shimalov and Shimalov 2003), whereas studies on infection of roe deer by blood sucking insects (*Lipoptena cervi*) (Valimaki et al. 2010) or bot flies (Nilssen et al. 2008) are published to a lesser extent.

In the Czech Republic, there are strict criteria for hunting deer. The hunting season is different for specimens as well for the sex of the deer. In the case of roe deer, bucks may be hunted only from May 16 to September 30 and does from September 1 to December 31. This situation complicates parasite research because is not possible to obtain the animals all year round.

For this reason, and with regard to the life cycle (primarily timing of life cycle), we can use only females for collecting *H.*

**Table 1** Prevalence, seasonal dynamics and annual intensity of infection of *Cephenemyia stimulator* larvae in roe deer monitored from 1999 to 2006 (mean number of larvae per head±standard deviation)

| Year | No. of animal examined | No. of positive animals | Prevalence (%) | The mean infection intensity in individual months | | | | | Number of larvae found per year | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | May | June | July | August | September | Mean | Minimum | Maximum |
| 1999 | 47 | 12 | 25.5 | 6±1 | 5.7±1.2 | 9±2 | 9.8±5.6 | 2±0 | 7.3 | 2 | 19 |
| 2000 | 107 | 38 | 35.5 | 10.1±5.9 | 8.2±4.4 | 6.7±2.7 | 7.6±3 | 6±2 | 8.3 | 2 | 22 |
| 2001 | 117 | 26 | 22.2 | 8.6±10.1 | 14±11.6 | 9.6±0.8 | 5.9±1.2 | 0 | 11.2 | 3 | 36 |
| 2002 | 72 | 23 | 31.9 | 10.7±5.9 | 11±12.3 | 5.5±6.9 | 3±0 | 0 | 8.6 | 1 | 21 |
| 2003 | 28 | 12 | 42.9 | 12.8±5.1 | 9.5±9.5 | 10±1 | 5±1 | 0 | 10.6 | 5 | 20 |
| 2004 | 55 | 19 | 34.5 | 12.3±1 | 9.2±3.2 | 8.3±3.9 | 6±0 | 4±0 | 9.9 | 3 | 30 |
| 2005 | 46 | 11 | 23.9 | 7.5±2.5 | 6±2.9 | 4.3±1 | 1±0 | 0 | 6.5 | 1 | 13 |
| 2006 | 31 | 5 | 16.1 | 6±1.1 | 7.3±2.5 | 3±0 | 0 | 0 | 6.2 | 3 | 10 |

*diana* larvae and males for *Cephenemyia stimulator*. To achieve greater result accuracy in the case of *H. diana*, only deer shot during the months of November and December were used in the study. During this time, the larvae are at the second stage. We believe that we can compensate for this disability with the number of animals as well as with repetition of the investigation over an 8-year period.

The observations of *Cephenemyia stimulator* by some authors from the Czech Republic have been reported (Dyk and Dyková 1962; Lamka et al. 1997; Vaca 2000). From other countries, investigations of nasal bots are rare, with few exceptions. In previous times in the Czech Republic, the prevalence ranged from 10 to 90 % (Lamka et al. 1997), later Vaca (2000) reported a low prevalence of 19 %.

In European countries, the prevalence of *Cephenemyia stimulator* is at approximately the same level as in the Czech Republic (32–43.2 % in France, Maes and Boulard 2000; 40.35–57.14 % in Slovakia, Čurlík, et al. 2004; 35.2 % in Hungary, Kiraly and Egri 2004). Kiraly and Egri (2007) later reported a prevalence of 11.1–76.9 % and mean intensities from 3.89 to 19 larvae per animal in 10 Hungarian counties.

The larvae of *Cephenemyia stimulator* develop gradually during the year, and once the larvae mature, the animals snort them out. In this study, the mean intensity of infection was highest in May and then gradually decreased until September (Table 1). Similarly, Barth et al. (1976) reported that the prevalence and mean intensity of nasal bots in roe deer in Germany tended to decrease between May and October, and similar results were published by Vaca (2000), whose study period extended from May to September. However, in Hungary, Kiraly and Egri (2007) discovered the highest number of larvae in August. Dudziński (1970a) and Papp and Szappanos (1992) also detected the most L3 larvae between April and August.

The main problem in observing *H. diana* larvae is that the period of subcutaneous does not coincide with buck hunting season (May 16–September 30). First instars of *H. diana* are very small up until the end of October and are very often overlooked as they measure up to 5 mm and migrate through the host tissues. For this reason, only does were investigated, and moreover, only does that were hunted from November 1 to December 31.

In this study, values of prevalence of *H. diana* ranged from 18.8 % in 2006 to 50 % in 2004; mean intensity of

**Table 2** Prevalence and intensity of *H. diana* infection

| Year | Number of larvae | | | | | |
|---|---|---|---|---|---|---|
| | No. of animal examined | No. of positive animals | Prevalence (%) | Mean | Minimum | Maximum |
| 1999 | 31 | 11 | 35.5 | 12.8 | 6 | 30 |
| 2000 | 50 | 13 | 26.0 | 14.1 | 4 | 31 |
| 2001 | 47 | 11 | 23.4 | 21.8 | 5 | 51 |
| 2002 | 56 | 13 | 23.2 | 15.4 | 3 | 50 |
| 2003 | 12 | 4 | 33.3 | 19.5 | 10 | 26 |
| 2004 | 30 | 15 | 50.0 | 18.2 | 2 | 42 |
| 2005 | 22 | 7 | 31.8 | 15.1 | 8 | 25 |
| 2006 | 16 | 3 | 18.8 | 15.3 | 13 | 18 |

**Table 3** The mean weight (in kilogram) and standard deviation of does infected and non-infected with *H. diana*

| Year | Infected animals | Non-infected animals |
|---|---|---|
| 1999 | 12.9±1.2 | 12.0±2.7 |
| 2000 | 13.2±1.6 | 13.2±1.4 |
| 2001 | 12.2±1.3 | 13.2±1.5 |
| 2002 | 12.5±1.0 | 12.4±1.3 |
| 2003 | 14.5±0.4 | 11.8±0.7 |
| 2004 | 13.4±1.1 | 12.9±0.7 |
| 2005 | 13.1±1.1 | 14.1±0.8 |
| 2006 | 13.7±1.3 | 14.3±0.8 |

infection ranged from 12.8 larvae in 1999 to 21.8 larvae in 2001. The results show that the prevalence of *H. diana* is very volatile and we cannot make accurate conclusions. Between 2000 and 2002, prevalence was relatively low, at around 25 %; it later increased to 50 %. The past 2 years have shown a downward trend, but it is questionable whether this trend will continue in future years.

Information on the prevalence of the *H. diana* from other countries is very sporadic, and often outdated. However, the reported prevalence is higher than in this study. In one research study conducted in Hungary, Egri and Husveth (2007) reported that *H. diana* larvae were found to occur in 84.3 % of roe deer, and Čurlík et al. (2004) reported a prevalence from Slovakia ranging from 51.72 to 60.61 %. Parasite infection as high as 100 % in Russia was recorded by Yamov et al. (1990), and in Bulgaria parasitism ranged from 48 to 66 % (Petrov et al. 1986). In Spain, Martinez-Moreno et al. (1994) described 90 % prevalence. In Slovakia, Čurlík et al. (2004) reported a mean intensity of infection rate of 64.4 larvae per animal.

The prevalence of both *Cephenemyia stimulator* as well as *H. diana* in the Czech Republic has gradually decreased since 1997. The situation has improved because the deer in the Czech Republic are treated each winter across the board with ivermectin. In areas where the treatment was not provided, anywhere from 50 to 85 % of roe deer were infected with bot flies. In hunting areas, where treatment was performed, prevalence dropped to 20 % (Lamka et al. 1996). Although the results showed a decrease in the number of parasites after treatment, it is evident that this treatment is only partially effective due to the fact that only some of the deer receive a sufficient quantity of feed.

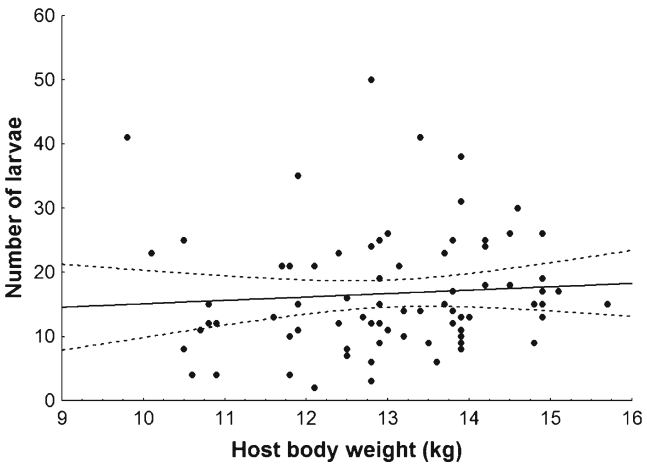


**Fig. 1** Plot of the regression between number of *H. diana* larvae and body weight in roe deer during 1999–2006

Many authors who have studied bot flies have also analysed the dangers the parasite poses toward the host. There were reports, especially in earlier years, of the lethal effects of these parasites (Grunin 1957, 1962). Vaca (2000) reports that roe deer infected by bot fly larvae lose 10–25 % of their body weight. Also, Sol et al. (2001) described the condition of the infested deer as poor. However, Dudziński (1970b) pointed out, in his detailed study of *Cephenemyia stimulator*, that deaths caused by parasitism should be cautiously investigated. Many deaths caused by other factors may be erroneously attributed to larvae of nasal bots; this is in the case where these larvae are found in large numbers in the dead animal. On the other hand, there is no doubt that parasites affect the quality of the roe deer population, disturb the process of feeding and assimilation of food, which hinders the animal's ability to gather sufficient amounts of fat for the winter period (Dudziński 1970b). Larvae of *H. diana* do not penetrate the internal organs of the host, and therefore they are less harmful. It is also known that many of these specific parasites and their hosts have created an internal control system during the course of evolution that regulates the number of parasites (Jahn et al. 2002). However, we cannot find any detailed studies on the biology of *H. diana* that include pathogenic effects. The authors describe only isolated cases of serious infection: Kettle and Utsi (1955), Ross (1983), Yeruham et al. (1994).

In this study, there were no conclusive differences found between the average body weights of parasite infected and non-infected deer. In many cases, the average weight of infected does was even higher. In Fig. 1, you can even see a faint tendency: the higher the larval count the higher the bodyweight. We believe that evaluating the actual effect of a parasite on a host animal is a complicated process. Overall health is influenced by other factors. The parasite is well adapted to its host, and the inflammatory process produced by larvae in the host organism is rarely observed. Jahn et al. (2002) discussed the complicated equilibrium the parasite shares with its host by means of the internal control system.

Due to the fact that only bucks were investigated with respect to *Cephenemyia stimulator*, and does with regard to *H. diana*, the results may seem somewhat inaccurate. However, we believe that the results are sufficiently informative. Male animals seem tend to have higher parasite loads than females in diverse species from salamanders (Anthony et al.

1994) to reindeer (Folstad et al. 1989). Also Dudziński (1970b) reported that males in the case of *Cephenemyia stimulator* are much more heavily infected. However, these facts have not been confirmed by extensive experimental study by Pollock et al. (2012).

Three important conclusions emerged from this study: (1) bot flies, *Cephenemyia stimulator* as well as *H. diana*, are common parasites in roe deer in the Czech Republic, (2) lower and medium levels of infection by *H. diana* (max 50 larva per animal) do not affect the weight of the animal.

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