# **Original papers**

# Cephenemyia stimulator (Diptera) infection in roe deer (Capreolus capreolus) from Kraków area, southern Poland

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**ABSTRACT.** The aim of the study was to determine the prevalence of botfly (Diptera: Oestridae) larvae infection in roe deer populations (*Capreolus capreolus*) in the Kraków area on the basis of necropsy and questionnaire surveys. Hunters were surveyed about the age and sex of hunted animals, and the origin of their habitat. All parasite specimens were identified to species in the laboratory. The parasites were found in the nasal cavities, esophagus, and larynx of male roe deer aged 3–8 years, living in forest habitats. The level of infection was relatively low (13%), with the intensity ranging from 1 to 10 larvae per host. Although no fly larvae were found during the dissection of roe deer carcasses, the parasites received from the hunters were identified as *Cephenemyia stimulator* botflies. These findings are supported by the evidence drawn from the questionnaires completed by the hunters.

Key words: parasites, Cephenemyia stimulator, Capreolus capreolus, necropsy, questionnaire study, Poland

## Introduction

One of the most abundant game species in Poland is the roe deer (*Capreolus capreolus*). The ungulate is common throughout the whole country, with its optimal habitat being the ecotone zone situated between forests and open areas (fields) [1]. The health status of roe deer depends on many factors, with one of the most important of these being parasitic diseases. Although endoparasites are well known to be present in *Capreolus capreolus* in Poland [2–5], there is little information on the occurrence of botflies from the genera *Cephenemyia* and *Hypoderma*. These parasites can cause health problems, resulting in the weakening of trophies (skulls), reduced carcass weight or even death; they can also affect the behavior of the host [6–7].

The aim of the study was to determine the prevalence of parasitic botfly (Diptera: Oestridae) infection in roe deer (*Capreolus capreolus*) living in the Kraków area on the basis of necropsy and a questionnaire survey.

## **Materials and Methods**

The studies were conducted over three years (2012–2014).

**Necropsy.** In total, 16 roe deer from the hunting areas located around Kraków were studied. The deer included two young does, seven young bucks approximately one year old, and seven adult bucks between one and eight years old. In order to check the presence of *Cephenemyia* sp. larvae, the nasal cavities were cleaved to allow examination of the frontal sinuses of the brain. The skin and spinal cord were visually inspected for the presence of *Hypoderma* sp. larvae.

Questionnaires were sent to hunters (n=100) to confirm the presence of these parasites during the preparation of the trophies (skulls) and skin of hunted animals. The survey included questions about the sex and age of the animals, location (hunting district), and the character of the environment (forest, meadow – open area, mid-

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forest meadow, or farmland). The location and number of botfly larvae per host (n=1-5, 5-10, more than 10) was also surveyed. All questionnaires were distributed with a tube containing 70% alcohol with glycerol for the collected parasites. The morphological identification of larvae was completed according to Zumpt [7] and Draber-Mońko [8].

#### Results

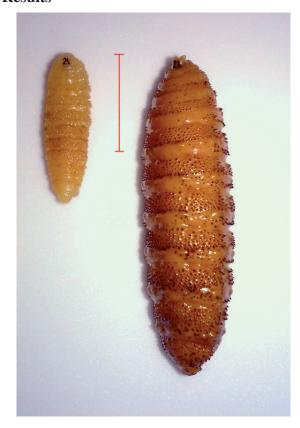


Fig. 1. Larvae of *Cephenemyia stimulator* at different developmental stages (scale bar = 1cm)



Fig. 2. Mouth hooks of *Cephenemyia stimulator* larva (scale bar =  $100\mu m$ )

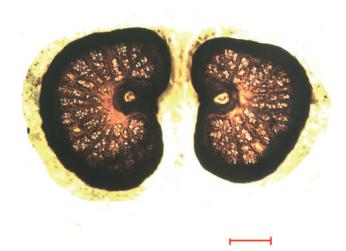


Fig. 3. Posterior peritremes of *Cephenemyia stimulator* III stage larva (scale bar = 100µm)

During the necropsy, no *Cephenemyia* larvae were found in the nasal or frontal sinuses, the throat or the brain of the roe deer examined. Likewise, no larvae from the genus *Hypoderma* were detected under the skin.

The examination of the received material, together with the questionnaires, confirmed the presence of *Cephenemyia stimulator* (Fig. 1–3) in 13 out of the 100 animals hunted. Only older bucks, aged three to eight years, were infected. The infected animals originated from a forest area near Myślenice (three cases), and the Niepołomicka Forest (ten cases). Between one and ten larvae, in the II and III stage, were found in the nasal cavities, esophagus or larynx of the examined animals.

#### **Discussion**

Although more than 40 species of flatworms (phylum Platyhelminthes) and nematodes (phylum Nematoda) are known to internally parasitize roe deer in Poland [9], little is known of infestation with ectoparasites (Diptera). The most common parasitic flies of roe deer can be classified into two families: the Oestridae and the Hippoboscidae [10–11].

The *Hypoderma* genus includes 11 species, five of which (*H. acetaeon*, *H. capreola*, *H. diana*, *H. moschiferi* and *H. tarandi*) are parasites of cervids. Although the life cycle of these parasites is poorly understood, it is believed to be similar to the developmental cycle of *Hypoderma bovis* (the bovine fly), a common fly in Poland which lives in the dorsal subcutaneous tissues of the host in their final stage [12].

The Cephenemyia genus includes nine species, of which three are responsible for cervid cefenomyiasis in Poland, namely: C. stimulator in roe deer, C. auribarbis in red deer and C. ulrichii in elk [8]. Recently, the presence of C. ulrichii was confirmed in elk from the Kampinoska Forest [13] and our present results confirm the presence of Cephenemyia stimulator in roe deer in southern Poland.

The life cycle of C. stimulator is probably similar to that of Oestrus ovis, the sheep fly. The viviparous females of this parasite gather near the hosts and inject larvae into their nostrils two weeks after copulation. From the nostrils, the larvae penetrate the nasal sinuses, larynx and pharynx, and sometimes migrate to the brain [7]. After about eight months of development, the larvae reach the third stage and are released through the nostrils into the environment, where they reach the imago stage and re-attack the host. C. auribarbis begins to infect the hosts (red deer) in May, while C. stimulator affects roe deer from June to late September [7]. Infestation with these parasites can lead to the death of the host as a result of difficulties in breathing and the intake of food.

It is also possible that the larvae can reach the brain of the host, and cause neurological diseases, as was described in a previous study on a female elk (Alces alces) in Oregon, USA which displayed uncoordinated movements, and showed signs of disorientation. The post-mortem examination revealed the presence of C. jellisoni larvae close to the left of the Eustachian tube [14]. This neurological effect was confirmed by the hunters interviewed in the present study, who observed that the roe deer infected by C. stimulator were less timid, and therefore easier to shoot (Prof Z. Bonczar, Institute of Animal Science, University of Agriculture, and Dr M. Wajdzik, Institute of Forest Ecology and Silviculture, University of Agriculture - unpublished data - oral information).

Cephenemyia botflies occupy primarily the temperate zone of the northern hemisphere [15], although species from this genus have possibly migrated to the palearctic ecozone. Cowell et al. [16] report the recent occurrence of two new species belonging to the genus Cephenemyia namely C. jellisoni and C. phobifera, in southern Canada. The spread of the botfly species may also occur through the transmission to new non-specific hosts. This phenomenon has been described elsewhere: for example, the larvae of C. ulrichii, considered to be

a specific parasite of elk, have also been found in the throat of roe deer in Finland [17].

As noted above, studies on this topic are rare in Poland, with most of these studies being performed in the 1960s and 70s. At that time, the larvae of Cephenemyia stimulator were found in 4% to 87% of roe deer, depending on the hunting area [6,18]. Dudziński [6] also confirmed the presence of these larvae in roe deer from the Kraków area; however, the mean intensity (32 larvae per host) and prevalence (85%) were significantly higher than observed in the present study. The presence of these parasites has also been reported in other neighbouring countries. Studies conducted during the period 1999-2006 in the Czech Republic, confirmed the presence of C. stimulator in 146 out of the 503 roe deer surveyed, with a prevalence of 28.8% and intensity of infection ranging from 6 to 11 larvae per host. During this investigation, another species of the Oestridae was found and subsequently classified as Hypoderma diana [19]. Research on the Cephenemyia botfly carried out in Spain found two fly species, C. auribarbis and Pharyngomyia picta, to coexist in red deer. Furthermore, it was observed that both species may inhabit an individual host because of the asynchrony of their respective life cycles, which effectively decreases the competition between them [20,21].

In other studies, a correlation has been found between the intensity of *C. stimulator* larva infection and the sex or age of the host. In contrast to our present finding, males were attacked more frequently than females, and young individuals were more susceptible to infection with larvae than older animals [20,21]. According to Dudziński [22], as it was observed in our study, larvae in developmental stages II and III may be derived from different populations of parasitic flies that are active during the warm months of the year. The results of our study confirm that only older males of roe deer were infected by *C. stimulator*.

C. stimulator infection can be treated using drugs containing ivermectin, but little is known of their effectiveness, and their application in free-living animals is difficult [19,23]. Further investigation in this area is needed in order to determine the effect of this parasite on the condition of the roe deer population in Poland.

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