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Case Report

Cephenemyia stimulator (Diptera: Oestridae) myiasis in a roe deer (*Capreolus capreolus*) from Portugal

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ABSTRACT

Herein we present a nasopharyngeal myiasis case by *Cephenemyia stimulator* in a roe deer hunted in Trás-os-Montes (NE Portugal). Preliminary inspection showed one larva sorting through the nostrils and the examination of the nasopharyngeal cavity showed more than 15 larvae in the glottis and retropharyngeal recesses. Four larvae were collected and stored into 70% ethanol for morphological and molecular identification. Three of the larvae were identified as third instars and the other one as a prepupa of *Cephenemyia stimulator*, being the first confirmation of this species in roe deer from Portugal. *C. stimulator* is currently widely distributed in roe deer from central and northern Spain so, the transboundary natural dispersal of these cervids would explain the introduction of this myiasis in Portugal. Further studies are needed to monitor the spreading of this infection in the westernmost populations of European roe deer.

1. Introduction

The roe deer (*Capreolus capreolus*) is one of the most abundant and widespread cervids in Europe. Its distribution ranges from Scandinavia to the Iberian Peninsula as its southwestern distribution limit (Apollonio et al., 2010). At the beginning of the 20th century, overhunting along with a substantial loss of forested areas caused by agricultural and pastoral activities, lead to the disappearance of this ungulate from almost all the Portuguese territory, with the exception of a few patches to the north of the Douro River. However, roe deer population size and density increased since the 70s, mainly due to a stricter hunting legislation and the reintroduction of individuals translocated from France and Spain for hunting purposes and also to increase prey availability for the endangered Iberian wolf (Torres et al., 2015). Natural colonization from nearby populations in north-western Spain was also recorded (Cardão, 2009).

Cephenemyiosis is an obligatory myiasis developing in the nasal and pharyngeal cavities of roe deer caused by *Cephenemyia stimulator* (Diptera: Oestridae) whose larvae are biontophagous, i.e., they feed on the living tissue of the host, producing inflammation of the nasal mucosa and an enlargement of the retropharyngeal recesses (Morrondo et al., 2021). In healthy animals, the presence of *Cephenemyia* spp. larvae rarely causes harm (McMahon, 1989).

C. stimulator is widely spread in the range of distribution of roe deer in Europe. In Spain, located at the southern range margin of distribution of this ungulate, the first case of cephenemyiosis in roe deer was reported in 2001 in an animal imported from France in 1997 to a fenced hunting area in Castilla-La Mancha (central Spain) and the first autochthonous infestation was observed in a roe deer from northwestern Spain in 2005 (Pajares, 2016). Nowadays, this myiasis is widely distributed in north and central Spain with an average prevalence and intensity of 62.2% and 41.2 ± 52.7 larvae per animal, respectively (Martínez-Calabuig et al., 2022).

The aim of this study was to present the first case of a nasopharyngeal myiasis by *C. stimulator* in Portugal and to discuss the possible origin of the introduction of this myiasis into the country.

2. Case presentation

In June 2021 a senior roe buck was hunted in the intersection

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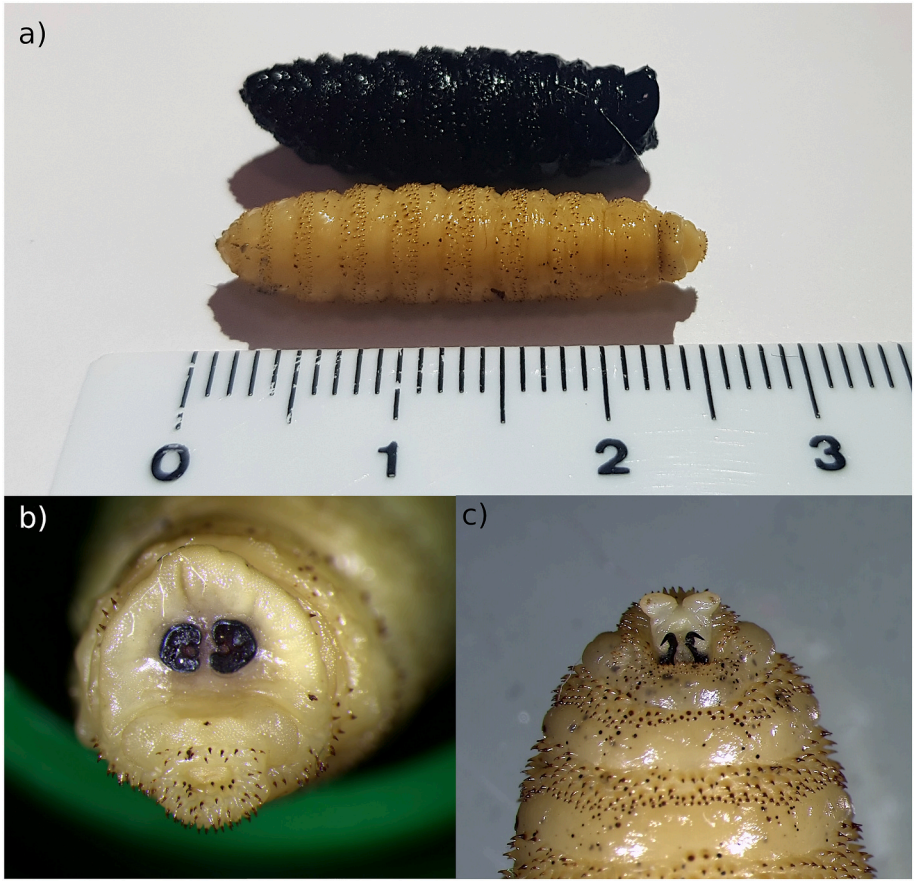

Fig. 1. (a) Third instar larva (yellow) and a prepupa (black) of *Cephenemyia stimulator* found in a roe deer hunted in Portugal. Note the pattern of distribution of the cuticular spines in the third instar (b). Posterior end of a third instar larva of *C. stimulator*. Note the reniform shape of the posterior spiracular plates. (c) Anterior end of a third instar larva of *C. stimulator* showing the typical V-shape disposition of the antennal lobes. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

between the districts of Bragança, Macedo de Cavaleiros and Vinhais (Trás-os-Montes, NE Portugal). The hunter who killed the animal referred that it displayed a poor body condition and a nervous behavior, snorting and running for no apparent reason.

Field preliminary examination of the animal showed one larva sorting through the nostrils and the examination of the nasopharyngeal cavity revealed more than 15 larvae in the glottis and retropharyngeal recesses. Most larvae were free on the mucosal surface and, only a few were firmly attached with their oral hooks. The larvae oriented their anterior end to the bottom of the pouches and the posterior spiracles towards the opening of the recesses. Apart from an enlargement of retropharyngeal recesses, no macroscopic lesions were observed around the larvae. Four larvae were collected and stored into 70% ethanol and submitted to the INVESAGA group for morphological and molecular identification.

Furthermore, this hunter (personal communication) reported another two cases of nasopharyngeal myiasis in the area, but no larvae were recovered so the infestation with *Cephenemyia* could not be confirmed.

Morphological identification for species and developmental stage was performed according to the keys of Zumpt (1965) and Quintela (2021), respectively. All the four recovered specimens were morphologically identified as *Cephenemyia stimulator*, according to the the distribution pattern of the cuticular spines (Fig. 1a), the reniform shape of the posterior spiracular plates (Fig. 1b), and the distance between the antennal lobes at the base of the antenna (Fig. 1c). Moreover, three of the larvae (24–26 mm long), showing yellowish to dark brown cuticle and dark pigmented peritremes, were classified as third instars and, the fourth larvae (22 mm long) with black cuticle was classified as a prepupa (Fig. 1a).

To confirm larval morphological identification, DNA was extracted from three larvae using a commercial kit (High Pure PCR Template Preparation Kit, Roche Diagnostics GmbH®, Mannheim, Germany) following the manufacturer's instructions. Oestridae DNA was detected by two PCR assays targeting the COX1 partial gene (Otranto et al., 2000) and the D2 fragment of the 28S RNA (Stevens and Wall, 2001). Amplicons of the expected size were purified and sequenced on an ABI 3730xl sequencer (Applied Biosystems, Foster City, CA, USA). Sequences were aligned and edited using ChromasPro (Technelysium, Brisbane, Australia) and consensus sequences were then scanned against the GenBank database using BLAST. Molecular identification confirmed the identification of *C. stimulator*. Unique sequences identified in this study were deposited in GenBank under accession numbers OQ791433-OQ791434 for COI and OQ793045 for 28S.

3. Discussion

Nasopharyngeal myiasis of roe deer is mainly the result of infection with larvae of bot flies of the genus *Cephenemyia*. All larval stages live on the surface of the host upper airways and, their bodies are covered with spines that, together with the oral hooks, serve to attach to the mucosa of the host. Affected animals manifest their discomfort through sneezing, coughing and sudden movements of the head trying to get rid of the larvae (Morrondo et al., 2021). Early larval stages of *Cephenemyia* spp. develops in the nasal mucosa of the host where they can enter in a phase of diapause in the winter months, whereas later stages invade the retropharyngeal recesses that distend from minute depressions of the pharyngeal wall into “pouches” or diverticula that can harbor up to 30 larvae (Cogley, 1987; Pajares, 2016). In this animal, all the larvae were finishing their development in the pharynx which coincides with the chronobiology described for this myiasis in Spain (Martínez-Calabuig et al., 2022) and other European countries (Király and Egri, 2007).

Despite the large size reached by the larvae, severe disease or death is unlikely because of the lack of involvement of surrounding tissues and

the ability of the nasopharynx to accommodate to greatly expanded retropharyngeal recesses (Cogley, 1987).

In this study, all the four recovered specimens were identified as *Cephenemyia stimulator*, being the first citation of this species in Portugal. Through the study of the COX1 partial gene all the obtained sequences were identical to each other and to the deposited sequences MN212908.1-MN212913.1; MN212915.1-MN212919.1 and MN212921.1 to MN212924.1 of *C. stimulator* obtained in roe deer from Spain (De la Fuente et al., 2021). These sequences also had a percentage of identity between 99.67% to 99.83% with the sequences of *C. stimulator* AF497768.1; NC_059850; MN212914.1; MN212915.1; MN212920.1; MN212925.1-MN212928.1 and MN212930.1 obtained in roe deer from France and Spain (Otranto et al., 2003; Aleix-Mata et al., 2021; De la Fuente et al., 2021). In addition, a 97.72% identity was observed with the deposited sequence AF497769.1 of *Cephenemyia trompe* obtained in a reindeer from Norway (Otranto et al., 2003). The sequences obtained through the study of the 28S RNA partial gene were identical to the reference sequences MN203990.1; MN203995.1 and MN203999.1 and had a percentage of identity between 99.16% to 99.72% with the sequences MN204000.1; MN204003.1-MN204006.1 and MN2040010.1-MN204011.1 of *C. stimulator* obtained in roe deer from Spain (De la Fuente et al., 2021). The percentage of identity observed with other *Cephenemyia* spp. deposited sequences such as *C. jellisoni* (KP954359.1; Winkler et al., 2015) or *C. auribarbis* (MN204016.1; De la Fuente et al., 2021) was lower than 95%.

Although roe deer specimens from France were reintroduced in central and south Portugal in the 1990s and early 2000s (Torres et al., 2015), *C. stimulator* infestations had never been reported in this country. The last documented reintroductions were performed in 2011 in Barrancos (South Portugal) and in 2013 in Arada mountain (Northwestern Portugal) with neighboring Spanish animals from Cádiz and León, respectively. However, the place where the roe deer was hunted is only 30 km from the northwestern Spanish border where this myiasis is known to be widely distributed (Martínez-Calabuig et al., 2022). The natural expansion from Spanish roe deer populations to Portugal has been documented (Cardão, 2009). Therefore, transboundary spreading of roe deer populations is the most probable hypothesis for explaining the introduction of this myiasis to Portugal.

Further studies are needed to monitor the evolution of this infection in the westernmost populations of European roe deer. A desirable approach would be necessary to involve hunters and game keepers in scientific research activities, to which they can and should actively contribute with their intellectual effort, their knowledge, or their tools and resources.

Declaration of Competing Interest

All the authors declared no conflicts of interest with respect to the research, authorship, and/or publication of this article.

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