



Prevalence of nasopharyngeal myiasis in roe deer (*Capreolus capreolus*) from an area with high sympatry between wild and domestic ungulates in Central Spain

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Abstract

Infestation by the sheep bot fly *Oestrus ovis* was firstly reported in a single roe deer from Central Spain in 2022. For assessing the current situation of nasal myiasis in this ungulate in this area, the nasopharyngeal cavities of 184 roe deer hunted in Central Spain between January–June 2023 were examined. All larvae were recovered and morphologically identified; in addition, species identification was molecularly confirmed in a subset of specimens. Forty-four roe deer (23.9%; CI 95 17.95–30.74) were positive for different Oestrinae larval stages. Twenty-six animals (14.1%; CI 95 9.44–20.02%) were infested by the roe deer nasal bot fly (*Cephenemyia stimulator*) with a mean intensity of 35.2 (SD 49.71) larvae/infested animal, and eighteen (9.8%; CI 95 5.90–15.02%) roe deer harboured the sheep bot fly (*O. ovis*), with a mean intensity of 2.0 (SD 1.33) larvae/infested animal. No mixed infestations by both Oestrinae were found in a single animal. All larval instars (L1, L2 and L3) of both species were identified. Most *C. stimulator* specimens were located at the nasal turbinates, and a small percentage (3.2%) at the pharynx; all *O. ovis* larvae were found at the nasal turbinates. Since *O. ovis* is highly prevalent in sheep and goat flocks from Central Spain, the high sympatry between roe deer and small ruminant populations in the studied area may have increased the risk of cross-infection. Moreover, the finding of mature L3 of *O. ovis* suggests that this species can complete its life cycle in roe deer. Therefore, monitoring bot flies in sheep and goat flocks as well as in sympatric wild ruminants is strongly recommended for achieving an optimum control of nasal myiasis.

Keywords Roe deer · Nasopharyngeal myiasis · Oestrinae · Central Spain · Sympatric ruminants

Introduction

Nose bot flies (Diptera: Oestridae) include nine genera infesting members of the orders Artiodactyla, Perissodactyla and Proboscidea (Scholl et al. 2019). They all belong to the subfamily Oestrinae which differ from other oestrids in that they are larviparous; gravid flies eject first instar larvae into the host nostrils which migrate to sinusal, frontal and/or nasopharyngeal cavities (Colwell 2001; Scholl et al. 2019) causing rhinitis, sinusitis, nasal discharge, and respiratory complications (Allen and Bunch 1982; Dorchie et al. 1998).

The most widely distributed and economically important nasal bot is *Oestrus* which develops within the nasal cavities and frontal sinuses of domestic and wild Bovidae (Zumpt 1965). Four *Oestrus* species are currently recognized: *O. ovis*, *O. variolosus*, *O. aureoargentatus* and *O. caucasicus*

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(Colwell et al. 2006). *Oestrus ovis*, known as the sheep bot fly, has a worldwide distribution in domestic sheep and goats, but it has also been described in wild ungulates such as the Alpine ibex (*Capra ibex*) (Zumpt 1965), the Argali (*Ovis ammon*) (Zumpt 1965), the Bighorn sheep (*Ovis canadensis*) (Capelle 1966) and the Asiatic ibex (*Capra sibirica*) (Sánchez et al. 2017). Human and carnivore infestations by *O. ovis* have also been occasionally described (Lobato 2011; Zanzani et al. 2016; Sante et al. 2017; Tabuenca-del Barrio et al. 2018).

In Spain, *O. ovis* prevalence rates in domestic sheep range from 70 to 84% (Alcaide et al. 2003, 2005; Gracia et al. 2010) and it has also been reported in wild sheep and goats such as European mouflon (*Ovis aries musimon*) (Moreno et al. 1999), Barbary sheep (*Ammotragus lervia*) (Barroso et al. 2017), and more recently in roe deer (*Capreolus capreolus*) (Martínez-Calabuig et al. 2023). In addition, a new *Oestrus* species has been detected in the Iberian ibex (*Capra pyrenaica*) that was tentatively identified as *O. caucasicus* (Pérez et al. 1996, 2016).

Cephenemyia (Latreille 1818) and *Pharyngomyia* (Schiner 1861) are the main genera of nasal bots infecting Cervidae in Holarctic regions. *Cephenemyia* exclusively parasitize deer from the Cervinae and Odocoileinae subfamilies (Buena de la Fuente et al. 1998). Four species in this genus (*C. ulrichii*, *C. auribarbis*, *C. stimulator* and *C. trompe*) infest cervids in the Palearctic region (Morrondo et al. 2021). The roe deer nasal bot, *Cephenemyia stimulator*, is very prevalent in the European roe deer (*Capreolus capreolus*), especially in Central Europe (Király and Egri 2007; Morrondo et al. 2021). In Spain, cephenemyiosis is a recent myiasis in roe deer. This species was firstly reported at the beginning of this century in one roe deer imported from France to Central Spain (Notario and Castresana 2001). Since then, cephenemyiosis has experienced a rapid expansion throughout the northern half of the Iberian Peninsula, with prevalences ranging from 31.6 to 62.2% and mean parasite burdens of 16.9–41.2 larvae/animal (Arias et al. 2016; Martínez-Calabuig 2022a).

Pharyngomyia picta, commonly known as deer throat bot fly, has been reported in deer from Europe and Asia (Colwell 2001). In Spain, it was found in the Iberian red deer (*Cervus elaphus hispanicus*), occurring commonly in mixed infestations with *C. auribarbis* (Ruiz and Palomares 1993; Buena de la Fuente et al. 1998; de la Fuente et al. 2000; Vicente et al. 2004) and it can also infest other sympatric wild cervids as fallow deer (*Dama dama*) (Ruiz and Palomares 1993). However, mixed infections by Oestrinae were not reported in roe deer, although a case of simultaneous infection by *C. stimulator* and *Lucilia caesar* (Diptera: Calliphoridae) has been detected in the nasal cavity of a single roe deer from northern Spain (Martínez-Calabuig et al. 2022b).

Since morphological features are quite similar between different dipteran species, especially among first instar larvae, molecular techniques are increasingly employed for diagnostic and taxonomic investigations of specimens belonging to this order. In this regard, the mitochondrial cytochrome oxidase subunit I (COI) has been used in several studies for assessing the host-specificity, species identification, phylogeny, and diagnosis of oestrid larvae (Otranto and Stevens 2006; Moreno et al. 2015).

Traditionally, oestrids have been considered as highly host-specific; thus, when larvae are introduced into a host widely different from their natural hosts, they do not usually complete their life cycle (Colwell 2006). However, *O. ovis* does not seem to fit in this theory, in view of the variety of hosts, as mentioned above, that can be infested by this species. In this regard, a recent investigation firstly reported *O. ovis* in Cervidae, specifically in one roe deer from the north of Guadalajara province in Central Spain (Martínez-Calabuig et al. 2023). It is worth noting that sympatry between wild and domestic ruminants is high in this area. After this first report, a broad survey was designed for providing updated information on the situation of nasal myiasis in roe deer from this area.

Materials and methods

Area of study and animals

The study was conducted in 35 game reserves located in five provinces of Central Spain (Guadalajara, Burgos, Soria, Segovia, and Zaragoza) from January to June 2023 (Fig. 1; Table 1). This area has a Continental climate characterized by cold winters and hot and dry summers. Precipitation is highly variable within and between years, ranging from scant to moderate and occurring mainly in spring and early summer (San Miguel et al. 2011; Charraza et al. 2018). Temperatures in summer usually exceed 35°C, favouring the lifecycle of oestrids, whose flies are active when temperatures are above 12–18°C (Breev et al. 1980). Dominant ecosystems in this area are the Mediterranean forest and grassland environments; the most abundant tree species are the oak (*Quercus faginea*), the holm oak (*Quercus rotundifolia*) and the Pyrenean oak (*Quercus pyrenaica*) (San Miguel et al. 2011).

According to the last official census, the study area comprises the Spanish regions (Castilla-La Mancha, Castilla-León, and Aragón) with the largest ovine populations (MAPA 2022). In addition, the abundance of roe deer in Central Spain is also among the highest of the country, as reflected by the high number of captures (average 7,000 roe

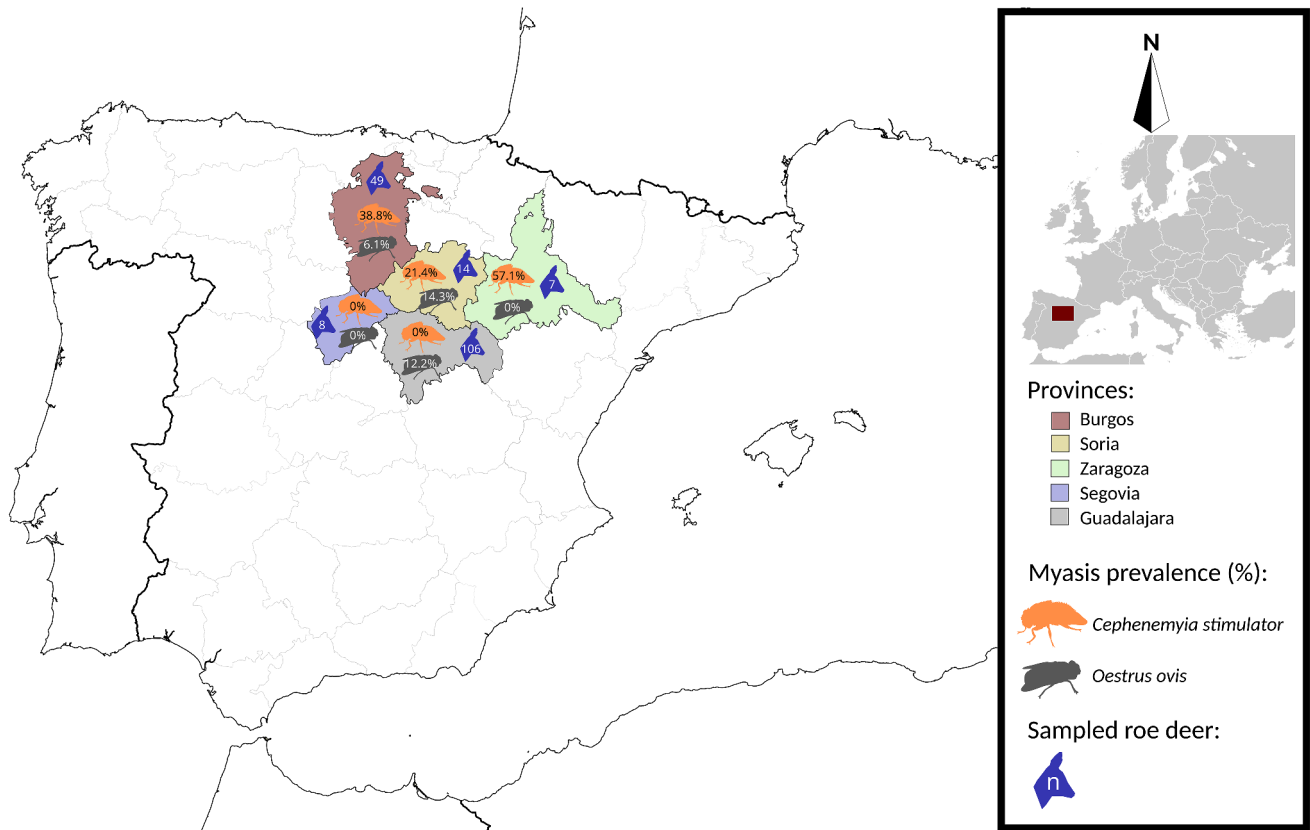


Fig. 1 Map of distribution of *O. ovis* and *C. stimulator* in roe deer from Central Spain

Table 1 Prevalence and intensity of infection by nasopharyngeal bots in roe deer (*Capreolus capreolus*) from Central Spain

Factor	Category	N ^o	<i>Oestrus ovis</i>		<i>Cephenemyia stimulator</i>	
			Positive (%)	Mean (SD)	Positive (%)	Mean (SD)
Province	Guadalajara	106	13 (12.2)	2.2 (1.28)	0 (0.0)	-
	Burgos	49	3 (6.1)	3.0 (2.00)	19 (38.8)	37.6 (54.59)
	Soria	14	2 (14.3)	3.0 (0.00)	3 (21.4)	63.0 (27.84)
	Zaragoza	7	0 (0)	-	4 (57.1)	3.0 (2.70)
	Segovia	8	0 (0)	-	0 (0.0)	-
Sex	Female	170	16 (9.4)	2.6 (1.31)	21 (12.4)	38.2 (51.46)
	Male	14	2 (14.3)	1.0 (0.00)	5 (35.7)	22.8 (44.34)
Age	Calf (< 1 year)	28	4 (14.3)	2.3 (1.50)	4 (14.3)	39.8 (42.67)
	Young (1–5 year)	106	6 (5.7)	2.8 (1.84)	14 (13.2)	31.9 (60.50)
	Old (> 6 year)	48	8 (16.7)	2.1 (0.84)	8 (16.7)	38.9 (34.62)
Month	January	12	1 (8.3)	3.0 (0.00)	1 (8.3)	33.0 (0.00)
	February	160	17 (10.6)	2.4 (1.37)	21 (13.1)	41.5 (53.29)
	March	2	0 (0.0)	-	0 (0.0)	-
	April	9	0 (0.0)	-	4 (44.4)	3.0 (2.71)
	May	-	-	-	-	-
	June	1	0 (0.0)	-	0 (0.0)	-
	TOTAL		184	18 (9.8)	2.0 (1.33)	26 (14.1)

deer) authorized in these provinces in the 2021/2022 hunting season (Centenera, unpublished data).

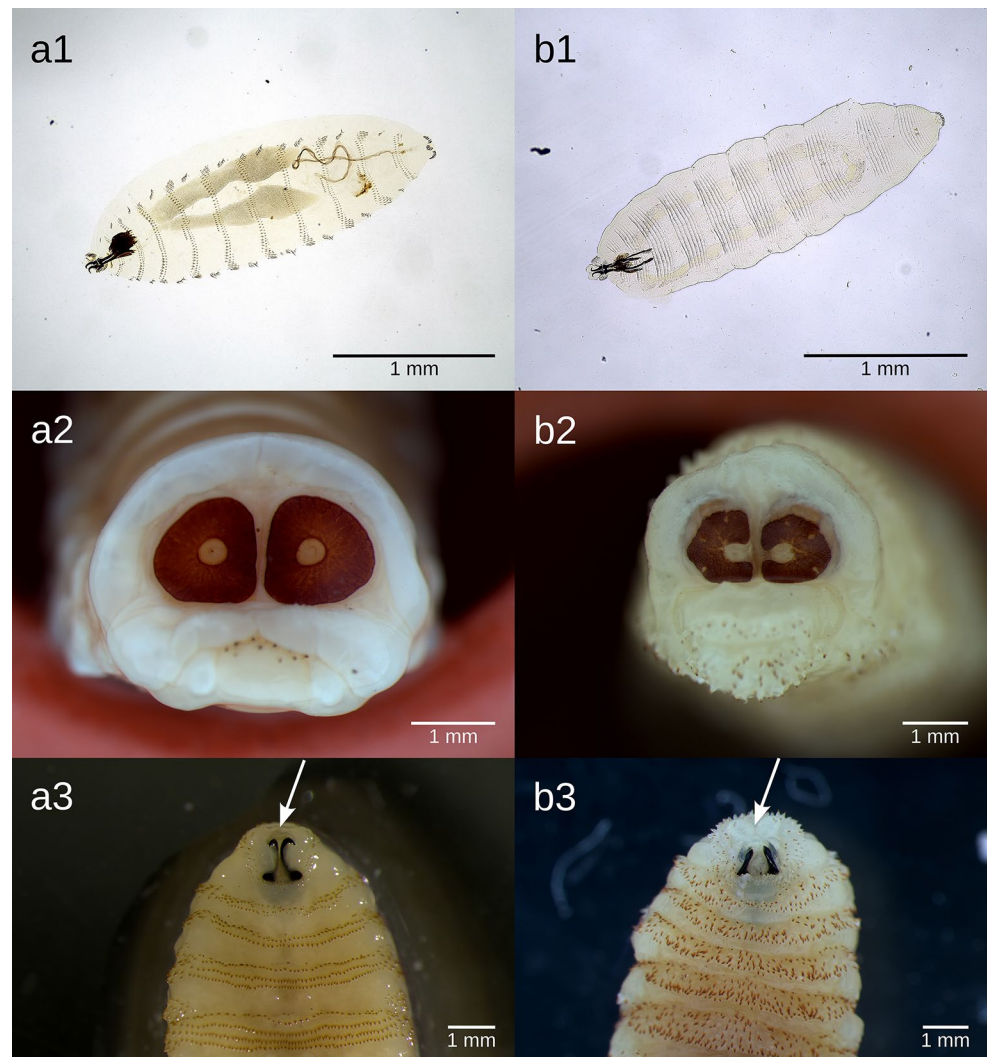
A total of 184 roe deer heads were collected in the field after hunting; they were stored at -20 °C until processed. Sampling was mainly restricted to January-February for

females and to April-June for males for coinciding with the official selective hunting season. Hunters' reluctance to relinquish the buck trophy resulted in a noticeable disproportion between sexes (170 females and 14 males). The age of roe deer was determined by teeth features (number, shape,

Table 2 Monthly distribution of larval stages of *C. stimulator* and *O. ovis* in roe deer from Central Spain

Month	<i>Oestrus ovis</i>			<i>Cephenemyia stimulator</i>		
	L1	L2	L3	L1	L2	L3
January	1	2	0	33	0	0
February	31	5	4	870	0	1
March	0	0	0	0	0	0
April	0	0	0	7	5	0
May	-	-	-	-	-	-
June	0	0	0	0	0	0
TOTAL	32	7	4	910	5	1

Fig. 2 (a) *Oestrus ovis* larvae. (a1) Detail of the cephalopharyngeal skeleton and the ventral spinulation pattern of a first instar (L1) showing two complete rows of spines in segments 1–4 and an additional incomplete row in the middle of segments 5–11. (a2) Detail of the posterior end of third stage larvae (L3) with D-shaped closed stigmal plates (a3) Detail of the anterior end of a L3 with antennal lobes separated at the basis (white arrow) and the typical spinulation pattern. (b) *Cephenemyia stimulator* larvae. (b1) Detail of the cephalopharyngeal skeleton and the ventral spinulation pattern of a L1 showing numerous rows of denticles on segments 2–11. (b2) Detail of the posterior end of a L3 with C-shaped stigmal plates (b3) Anterior end with V-shaped antennal lobes (white arrow)



and wear of the dental pieces) according to Høye (2006), and three host age categories were considered (< 1 year; 1–5 year; > 6 year).

Larval collection and identification

Roe deer heads were thoroughly examined for nasopharyngeal myiasis according to Martínez-Calabuig (2020). All larvae found in the nasopharyngeal cavities were recovered, rinsed in physiological saline, and preserved in 70%

ethanol. The number, location and stage of the larvae were recorded (Table 2). Morphological identification was performed according to Zumpt (1965). First instars were identified according to the shape of the anterior cephalopharyngeal skeleton and the spinulation pattern (Fig. 2a1, b1); identification of L2 and L3 was carried out on the basis of the shape of posterior spiracular plates (Fig. 2a2, b2), antennal lobes disposition and spinulation pattern (Fig. 2a3, b3).

Morphological identification of both species was molecularly confirmed by the analysis of a subset of larvae

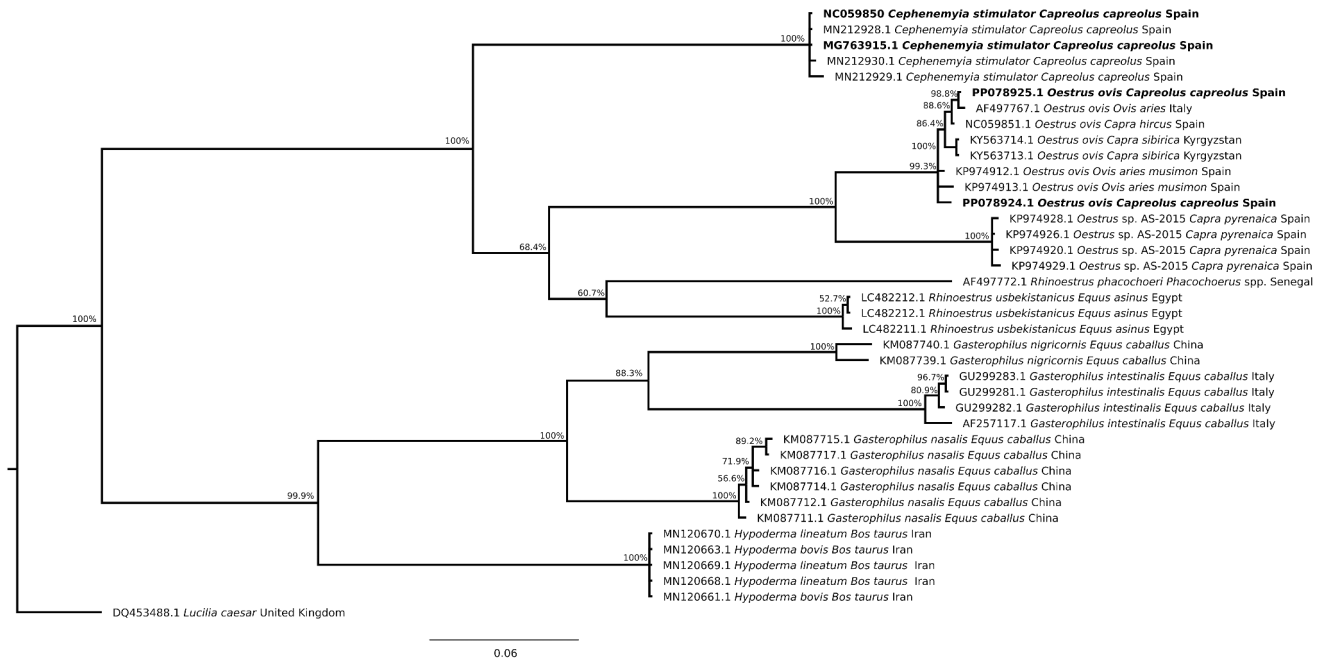


Fig. 3 Phylogenetic tree clustering of the partial COI of Oestridae. The tree was obtained using a General Time Reversible substitution model with gamma-shaped rate variation with a proportion of invariable sites (GTR+G+I) with MrBayes software 3.2.7 (Ronquist et al. 2012), using Bayesian inference with Markov Chain Monte Carlo sampling

including specimens of the three larval instars. DNA was extracted using a commercial kit (High Pure PCR Template Preparation Kit, Roche Diagnostics GmbH®, Mannheim, Germany) following the manufacturers' instructions. DNA samples were analysed using a PCR targeting a 688 bp segment of the mitochondrial COI gene of Oestridae as previously described (Otranto et al. 2000); DNA of *Hypoderma actaeon* and nuclease free water were included as positive and negative controls, respectively.

PCR products were separated by electrophoresis on 1% agarose gels stained with RedSafe (iNtRON Biotechnology®, South Korea) and visualized using a GelDoc Go Imagen System (Bio-Rad Laboratories®, California, USA). Selected fragments were purified and sequenced at STAB VIDA's laboratories of the Universidade Nova de Lisboa, Portugal. Sequences were aligned and edited using ChromasPro (Technelysium, Brisbane, Australia) and consensus sequences were then scanned against the GenBank database using BLAST.

Phylogenetic analysis

A phylogenetic analysis was carried out using MrBayes 3.2.7 software (Ronquist et al. 2012) by Bayesian approach with Markov Chain Monte Carlo sampling (10,000,000 generations sampling every 1,000 steps). A General Time Reversible substitution model with gamma-shaped rate variation

(10,000,000 generations, sampling every 1,000 generations). This analysis involved 39 nucleotide sequences. The nucleotide sequence of *Lucilia caesar* was used as an outgroup. Isolates obtained in this study or identical to those obtained in the present study are highlighted in bold

with a proportion of invariable sites (GTR+G+I), was used. The model was selected based on AIC value (Akaike Information Criterion) using the free software jModelTest v.2.1.10 (Darriba et al. 2012). The tree was visualized and edited using FigTree 1.4.3 (<http://tree.bio.ed.ac.uk/software/figtree/>).

Statistical analysis

Statistical analyses were performed with R statistical package (R v.4.3.2; 3). Variables were grouped and categorised for statistical analysis as represented in Table 1. A logistic regression analysis was performed for assessing the possible influence of different factors (age, sex, province, and month of capture) on the prevalence of *O. ovis* and *C. stimulator*. The binomial GLM was fitted with the brglm2 package (Kosmidis 2023). Identification of risk factors affecting the intensity of infestation was assessed using the ANOVA test. Since larval counts were not normal distributed, they were log normalized (natural log + 1) previous to the analysis. Only data from positive animals were used for performing the ANOVA test.

Results

Overall, 44 out of 184 (23.9%; CI 95 17.95–30.74) roe deer heads harboured dipteran larvae ($n=959$) in their nasopharyngeal cavity, and the mean number of larvae per infested roe deer was 35.2 (SD 49.71).

Morphological examination allowed the identification of two Oestrinae species: *C. stimulator* and *O. ovis* (Fig. 2); these findings were molecularly confirmed. Regarding *O. ovis*, COI sequences showed a percentage of identity between 98.9 and 99.8% when compared to the deposited sequences NC_059851.1 and AF497767.1 of *O. ovis* larvae recovered from sheep and goat from Spain and Italy (Otranto et al. 2003; Aleix-Mata et al. 2021). The phylogenetic analysis revealed that our sequences clustered with other *O. ovis* sequences obtained in Spain, Turkey, and Kyrgyzstan, being clearly separated from a novel *Oestrus* species detected in an Iberian ibex from Spain (Moreno et al. 2015) (Fig. 3). In addition, the COI sequences of *C. stimulator* were identical to those (MG763915.1 and NC_059850.1) recovered from roe deer from Spain (Aleix-Mata et al. 2021; Fidalgo et al. 2021); the phylogenetic tree showed that all these sequences clustered with other sequences of *C. stimulator* recovered from roe deer in our country (de la Fuente et al. 2021). The partial sequences obtained in the present study were deposited under the accession numbers PP078924.1–PP078925.1.

Cephenemyia stimulator was the most prevalent nasal bot fly since 26 animals (14.1%; CI 95 9.44–20.02%) were infested, showing a mean intensity of infestation of 35.2 (SD 49.71) larvae/infested animal; after identification of larval instars, 910 were classified as L1, five as L2 and one as L3 (Table 2). Only eighteen roe deer (9.8%; CI 95 5.90–15.02%) harboured *O. ovis*, and the mean intensity of infestation was 2 (SD 1.33) larvae/infested animal; the distribution of the different larval stages was 32 L1, 7 L2 and 4 L3. No mixed infestations by both Oestrinae were found in any animal. Table 2 shows a strong predominance of first instars (> 65%) for both species, especially in January and February. Regarding to the location of larvae, all *O. ovis* specimens ($n=43$) were located at the nasal turbinates; those of *C. stimulator* ($n=916$) were also mainly found at the nasal turbinates ($n=884$; 96.8%), although a small number was detected at the pharynx ($n=32$; 3.2%).

Prevalence and intensity of infestation values considering the different factors analysed are summarized in Table 1. *Oestrus ovis* was detected in the provinces of Guadalajara, Burgos and Soria with percentages ranging from 6.12 to 14.29%. Moreover, *C. stimulator* was found in roe deer from Burgos, Soria and Zaragoza, reaching higher prevalences (35.7–57.1%) than *O. ovis*. It is worth noting that both Oestrinae were detected in roe deer from Burgos and Soria, although no mixed infestations were found in any

animal. On the contrary, both species were absent in the eight roe deer hunted in Segovia.

Regarding the sex of the hosts, the prevalence for both species was higher in males than in females. In general, old animals (> 6 year) showed high prevalence values than young adults and calves; however, the logistic model over *O. ovis* and *C. stimulator* prevalence did not show any statistical differences when considering the sex, age, month, or province of origin ($p > 0.05$).

The influence of the month when roe deer were hunted was only significant for the intensity of infestation by *C. stimulator* ($p=0.004$), with higher larval burdens in February than in April.

Discussion

Two different nasal bot flies, *C. stimulator* and *O. ovis* are currently affecting roe deer in central Spain. Our results reveal that the prevalence and intensity of *C. stimulator* in roe deer from this area is much lower (16.3%) than that previously detected by Martínez-Calabuig et al. (2022b) in northern Spain. Nevertheless, our data show high variations between provinces that ranged from 0% in Guadalajara and Segovia to 57.1% in Zaragoza (Fig. 1). Marked differences in the prevalence of *C. stimulator* among neighboring provinces, such as Zaragoza and Guadalajara or Segovia and Burgos, are probably due to the current irregular distribution of this myiasis within those provinces; thus, all positive animals from Zaragoza were hunted in the northern part of the province, while those closest to the border with the province of Guadalajara were negative. Similar findings were observed in roe deer from Segovia and Burgos since most of the positive animals from Burgos belonged to the northernmost populations located far from Segovia province. In addition, the noticeable prevalence found for the sheep bot fly, *O. ovis*, suggests that its presence in roe deer was not accidental as it had been hypothesized after its first identification.

Oestrosis is extensively distributed in domestic and wild sheep and goats in Spain (Alcaide et al. 2003, 2005; Gracia et al. 2010). Nevertheless, there have been widespread reports of accidental infestations in other species, including humans and dogs (Lucientes et al. 1997; Moreno et al. 1999; Lobato et al. 2011), suggesting that gravid *O. ovis* females are not strictly host specific (Colwell 2001). According to our findings, the occurrence of late larval stages (L2–L3) in roe deer suggests that *O. ovis* could complete its life cycle in Cervidae.

Alcaide et al. (2005) indicated that high *O. ovis* prevalence and intensity of infection values in its main hosts (domestic small ruminants) may potentially increase the

risk of spillover of this parasite to new hosts. High roe deer densities (> 10 animals/100 ha) and the existence of shared habitats with abundant sheep flocks in the area of study may have favoured the interspecific spillover of *O. ovis*. A similar situation has been previously described for *Hypoderma actaeon* (Diptera: Oestridae), considered specific for red deer, which has become an emerging myiasis in roe deer in Central Spain (Panadero et al. 2017). Changes in the pattern of distribution of potentially susceptible hosts (red deer/roe deer) in Central Spain may have favoured the spreading of this myiasis to other hosts (Panadero et al. 2020). It is worth noting that some authors (Moreno et al. 1999) consider oestrid flies as host-opportunistic so that their specificity would be very influenced by the availability of adequate host species (Price 1980). Our results would reinforce the lack of host specificity of *O. ovis*.

Although our study was restricted to the first semester of the year, our results show a strong predominance of first instars for both Oestrinae species from January to April, indicating the existence of a hypobiotical period where larvae delay their development while waiting for the optimal breeding season (Alcaide et al. 2003). Additionally, the existence of a low proportion of L2 and L3 during this period would reveal the end of the quiescence period and the beginning of larval reactivation. However, a year-round sampling, covering the entire life cycle of these parasites is needed for a complete analysis of their chronobiology.

Larval burdens, mainly represented by first instars, were much higher for *C. stimulator* than for *O. ovis*. Taking into consideration that L1 represent the insurance of the survival of the parasitic population (Tabouret et al. 2001), those differences could be attributed to the major suitability of roe deer as a host for *C. stimulator* than for *O. ovis*. In this sense, several factors promoting larviposition and subsequent larval survival have been related to the suitability of a host for Oestrinae, e.g. host related odour (Poddighe et al. 2010), moistness (Cepeda-Palacios et al. 2000) and structure of the host's muzzle (Cogley and Anderson 1981), host immune reaction (Tabouret et al. 2003), as well as behavioural responses to avoid larviposition (Anderson 1975).

Oestrids have very different life cycles and adaptations for survival at different sites into the host (Colwell et al. 2006). Early larval stages (L1) of *C. stimulator* are found in the nasal cavity of roe deer, whereas late stages (L2-L3) are mainly located in the pharyngeal pouches or diverticula (Angulo-Valadez et al. 2010; Martínez-Calabuig 2020). In this study, all *O. ovis* larvae were only found in the nasal passages of roe deer, whereas in its natural hosts, sheep and goats, larvae are found in the nasal cavity as well as in nasal and frontal sinuses (Angulo-Valadez et al. 2010).

Statistical analysis did not allow identifying any factor significantly affecting the prevalence of infestation by *C.*

stimulator and *O. ovis*. The absence of significant differences may be due to the low number of roe deer infested within some categories.

Our results also reveal that the geographical distribution of both Oestrinae in roe deer from Central Spain is not homogeneous. *Cephenemyia stimulator* is a high prevalent myiasis which is actually present in three out of the five provinces surveyed in this study, whereas *O. ovis* was only sporadically found in three provinces. It is worth noting that the infestation by *O. ovis* was more prevalent in the province of Guadalajara, where *C. stimulator* is absent, than in Burgos and Soria, where both species coexist; nevertheless, it is worth noting that in the latter provinces, no roe deer was simultaneously parasitized by both species. Further studies to elucidate the influence some biotic (host density) and abiotic (climate, altitude, etc.) factors in the distribution of nasal myiasis are needed.

The absence of co-occurrence within the same individual could be attributed to behavioural changes of roe deer after being attacked by *C. stimulator* flies. In this sense, Anderson (1975) evidenced that, after a first infection by *Cephenemyia* spp., experienced deer try to evade larviposition by oestrid females, also reducing the infection success by *O. ovis*. Although co-occurrence of different oestrids such as *P. picta* and *C. auribarbis* are commonly reported in red deer from Europe (Ruiz and Palomares, 1993; de la Fuente et al. 2000; Vicente et al. 2004; Leitner et al. 2016; Miranda et al. 2022), the earlier larviposition by *C. auribarbis* and its faster larval development compared to *P. picta* in southern Spain may reflect asynchronous life cycles of both oestrids decreasing the co-occurrence of both sympatric species (Bueno-de la Fuente et al. 1998). In addition, it was reported that the intensity of *P. picta* in concomitant infections with *C. auribarbis* was lower than in pure infections (Vicente et al. 2004), providing good evidence of interspecific competence, which could be dealt with by parasites by means of asynchronous life cycles and different maturation periods.

Conclusion

Our data suggest a possible interspecific transmission of *O. ovis* from domestic ruminants to roe deer in central Spain; this may be due to the high density of this wild ungulate and free ranging sheep which usually share habitats in this area. Thus, this Spanish region may be considered a hotspot for cross-transmission of different Oestridae between Cervidae and Bovidae, as it has been previously reported for *H. actaeon*. Since wildlife may contribute to the reinfestation of domestic flocks with *O. ovis*, the efficacy of oestrosis control programs can be compromised. Thus, monitoring

strategies must include sheep and goat flocks together with sympatric wild ruminants.

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Author contributions NMC wrote the main manuscript and investigation; RP, PD conceptualization, supervision, review and editing; GV, AS, DGD investigation and methodology, SR, CL prepared figures, supervision, review and editing; PDB, PM conceptualization and funding acquisition.

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Data availability The datasets generated during this study will be available upon request.

Declarations

Ethical approval Samplings were performed on animal carcasses obtained through legal hunting activities without research purposes, so authorization from the bioethics committee was not required.

Competing interests The authors declare no competing interests.

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