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Pedicle myiasis by *Lucilia caesar* (Diptera, Calliphoridae): An emerging disease in roe deer from north-western Spain

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Abstract

A total of 35 male roe deer (*Capreolus capreolus*) from Lugo province (north-western Spain) were examined for pedicle myiasis between 2020 and 2022. All these animals had died by different causes and were examined or preserved just after their death to avoid post-mortem infestations. After external and internal head inspection, five animals were diagnosed with severe myiasis at the basis of the antlers (14.29%; 95% confidence interval = 5.38–31.04). All the affected bucks presented a cutaneous wound (1.5–15 cm in diameter) around the pedicles with extensive tissue destruction, exposition of frontal and parietal bones, and massive infestation by dipteran larvae (2–12 mm long). Four of five roe deer showed whitish egg clusters adhered to the basis of the antlers. Moreover, in one animal, six larvae had penetrated the cranial cavity, and in two of them larvae were also found in the nasopharyngeal cavity. All the cases were recorded in summer (May–August). Morphological identification and subsequent molecular confirmation revealed that all animals were infested by different larval stages of *Lucilia caesar* (Diptera: Calliphoridae). This study represents the first report of pedicle myiasis in Spain. Since this disease is reported sporadically, the detection of five cases in a short period of time suggests an increase in the incidence of this myiasis.

KEYWORDS
Calliphoridae, *Capreolus capreolus*, *Lucilia caesar*, north-western Spain, pedicle myiasis

INTRODUCTION

Blowflies (Diptera: Calliphoridae) include some of the most economically significant parasites of livestock worldwide, causing facultative myiasis (Lihou & Wall, 2019). Although most calliphorids are secondary flies that follow a pre-existent infestation, a small number of species such as *Lucilia cuprina* (Wiedemann, 1830), *Lucilia sericata* (Meigen, 1826) and *Lucilia caesar* (Linnaeus, 1758) can produce myiasis without previous existing injury (Zumpt, 1965). Blowflies are characterised by a rapid larval feeding stage, mainly in cutaneous infestations, and high pathogenicity (Stevens et al., 2006). The establishment of the first stage larvae (L1) on the dermis is facilitated by the excretion of digestive proteases that are even capable to digest bone sutures (Sandeman et al., 1987).

A wide range of wild and domestic vertebrates may suffer from blowfly myiasis, demonstrating low host specificity. Hosts became infected when gravid female flies, attracted by soiling of the moist wool or hair, wounds, or inflamed skin, lay eggs on a previously existing lesion (Broadmeadow et al., 1984; Sandeman et al., 1985). Almost any type of wound can become infested with calliphorid larvae, including those caused by management practices such as castration, dehorning, branding, shearing, and so forth. Moreover, it has been reported that gravid *Wohlfahrtia magnifica* (Diptera: Sarcophagidae)

TABLE 1 Data and main findings in the roe deer bucks diagnosed with pedicle myiasis in Lugo province, NW Spain (2020–2022).

Roe deer	Date	Municipality	Death	Age (year)	Wound diameter (cm)	Location of Calliphorid larvae (number)	Eggs[a]
1	Jl/20	Palas de Rei	Car crash	1.5	2	Around the pedicles (80) Nasal cavity (1)	++
2	Jl/21	Lugo	Euthanised	>6	15	Around the pedicles (>600) Cranial cavity (6) Nasal cavity (5)	+++
3	Jl/21	Viveiro	Hunted	3	10	Around the pedicles (>200)	+++
4	Au/21	O Corgo	Euthanised	4–5	1.5	Around the pedicles (44)	–
5	My/22	Pobra do Brollón	Euthanised	2	1.5	Among the pedicles (250)	+

[a]–nil; +mild; ++moderate; +++intense.

flies are strongly attracted to some chemical substances present in sexual fluids (Farkas et al., 1997; Ruiz-Martínez et al., 1987). It has been demonstrated that head-butting during dominance fights between males often results in wounds that can be easily infested by flies (Sotiraki et al., 2003). Consequently, in rams, the head is the second most frequently infested body region after the genitalia (Remesar et al., 2021; Sotiraki et al., 2005). Cervid antlers bleed easily during the velvet stage. Afterwards, a rise of androgen concentrations leads to the loss of the skin covering the antler, which is polished in preparation for the mating season. During both periods, the presence of blood and dead tissue attracts gravid flies that may deposit their eggs on the basis of the antlers. Therefore, newly hatched larvae can invade the tissues around the pedicles.

There is a lack of previous reports of pedicle myiasis in cervids from Spain. In north-western Spain, roe deer (*Capreolus capreolus*, Linnaeus, 1758) is the most abundant wild ruminant, and their population has significantly increased in size in the last two decades (Markina, 2017). In 2020, a first case of pedicle myiasis was observed in a roe deer from Lugo province (northwest Spain). From this first detection until 2022, roe deer were examined for investigating the incidence of this myiasis and for determining its causes.

MATERIALS AND METHODS

Animals and study area

The study was carried out in Lugo province (north-western Spain, 43°0′ 44″ N, 7°33′ 21″ W), which is one of the Spanish geographical areas with the highest density of roe deer. This province is characterised by the presence of large areas of woodland, mainly composed of *Quercus robur* and *Castanea sativa* (Ortega, 2009) and the climate is temperate oceanic, with mild, rainy winters and warm and relatively sunny summers.

Between 2020 and 2022, a total of 35 roe deer bucks were submitted to the INVESAGA Laboratory at the School of Veterinary Medicine in Lugo as part of a project for studying the prevalence of nasopharyngeal myiasis by *Cephenemyia stimulator* (Diptera: Oestridae) in roe deer. All the animals, except one whose head was provided directly by a hunter, were collected by the Natural Heritage Service in a moribund condition or recently dead. The cervids were immediately examined or preserved at −20°C to avoid post-mortem infestations. The age of the animals was determined according to their teeth features (Høye, 2006).

Roe deer head examination

Roe deer heads were thoroughly examined, particularly around the base of the antlers, to find skin wounds, ulcerations and ectoparasites, paying special attention to the presence of dipteran larvae. Moreover, the presence of dipteran eggs on the wounds or antlers was also recorded and a qualitative estimation of the number of eggs, from nil (–) to intense (+++), was established (Table 1). The nasal and cranial cavities were subsequently opened and examined for assessing the presence of dipteran larvae. All the detected larvae were recovered, counted and stored in 70% ethanol for further identification.

Morphological and molecular identification

Larvae were identified under the stereomicroscope using morphological keys (Zumpt, 1965). Since accurate morphological identification can be difficult in some Calliphoridae species, molecular identification was carried out in a subset of larvae from each animal. DNA was extracted using a commercial kit (High Pure PCR Template Preparation Kit, Roche Diagnostics GmbH®, Mannheim, Germany) following the manufacturer's instructions. DNA was subjected to two PCR protocols targeting a partial region of the genes encoding for the 28S RNA (Stevens & Wall, 2001) and the c oxidase subunit I (*cox1*) (Otranto et al., 2000).

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 scanned against the GenBank database using BLAST.

RESULTS

Five cases of pedicle myiasis were detected in 35 roe deer analysed between 2020 and 2022 (14.29%; 95% confidence interval = 5.38–

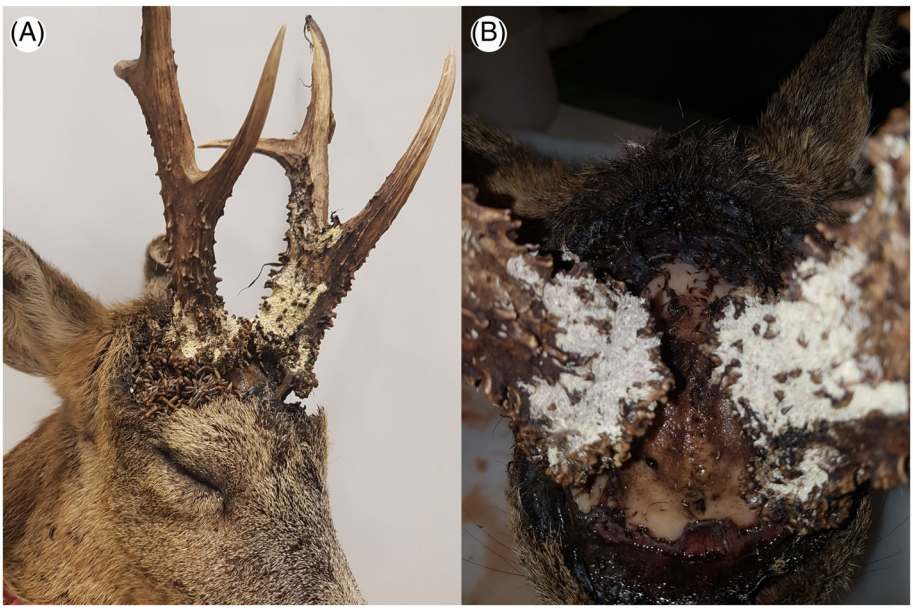

FIGURE 1 Roe deer #2 showing (a) clusters of dipteran eggs stuck to the basis of the antlers and to the remains of the velvet and a high number of *Lucillia caesar* larvae around the pedicles; (b) note the circular wound that left exposed a large area of the skull.

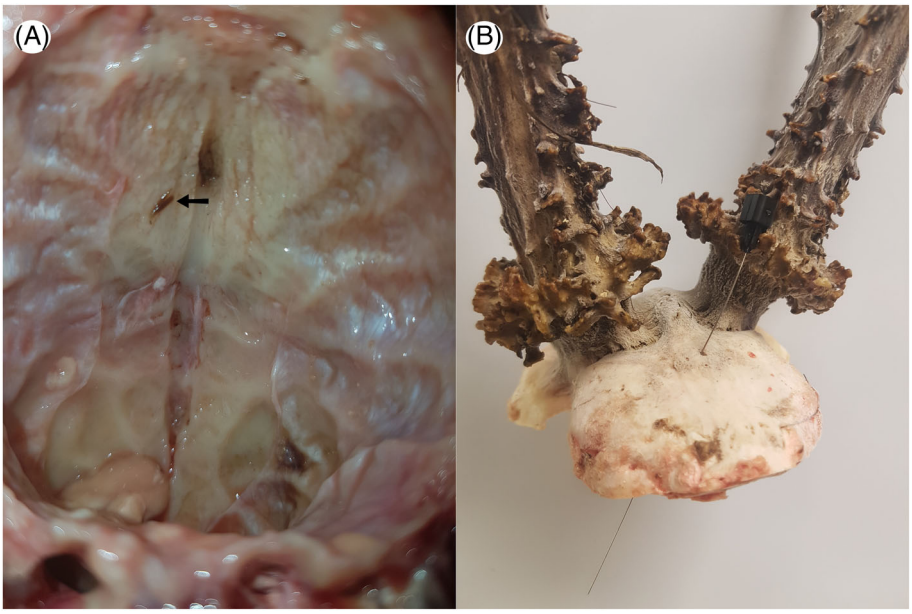

FIGURE 2 (a) Larva of *Lucilia caesar* on the meninges of roe deer #2. (b) Orifice in the cranial suture through which six larvae entered in the cranial cavity.

31.04). All cases were recorded in summer (May–August) in bucks aged from 1.5 to 6 years. The main data and findings observed in each animal are summarised in Table 1.

The external inspection of the head of the affected bucks revealed the presence of a deep wound in the proximity of the pedicles showing a high number of blowfly larvae (2–12 mm long) embedded in putrid tissue (Figure 1). Two animals (RD2 and RD3) exhibited a large circular wound (10–15 cm diameter) around the pedicles exposing a large area of the skull (Figure 1b), whereas the rest of the bucks presented a cutaneous orifice (1.5–2 cm diameter) next to the pedicles through which the larvae penetrated, causing a large and heavily infested subcutaneous wound. Moreover, four of the five roe deer showed whitish spots stuck to the basis of the antlers that were identified as masses of dipteran eggs (Figure 1a,b).

Internal examination of nasal and cranial cavities revealed that two animals (RD1 and RD2) showed a mixed nasal infestation with Calliphoridae and *Cephenemyia stimulator* larvae. One roe deer (RD2) presented five larvae on the meninges (Figure 2a) and a single larva

crossing through a cranial suture (Figure 2b). No brain lesions were macroscopically observed. Morphological identification and subsequent confirmation by PCR showed that all the affected animals were infested by different larval stages of *Lucilia caesar* (Diptera: Calliphoridae). Sequence analysis at the partial 18S RNA and *cox1* showed that the obtained sequences were identical to the deposited sequences MF694304 and KM657113.1 of *Lucilia caesar* (Linnaeus, 1758).

DISCUSSION

The present study reports the detection of pedicle myiasis in 5 of 35 roe deer examined during a 3-year period in north-western Spain. Those findings are remarkable since the number of reports about this disease in wildlife is scarce. To our knowledge, only two cases of pedicle myiasis by Calliphoridae have been published in wild deer up to now. The first outbreak was reported by the Duke of Bavaria (de Bavière, 1983) in the Styrian region of Austria in the 70s, which caused the death of the biggest bucks. More recently, Nielsen et al. (2010) diagnosed four cases of severe pedicle myiasis in roe deer from Sweden.

Pedicle myiasis in cervid hosts is considered to be predisposed by injuries near the pedicle caused by fights during the rutting season or secondary to scratching for detaching the velvet (Azrolharith et al., 2022). In the cases reported herein, the existence of previous cutaneous injuries could not be proven due to the advanced state of the myiasis. Nevertheless, the fact that most of the animals presented eggs attached to the antlers, reinforce the hypothesis that flies were attracted to the smell of the dead tissue adhered to the antlers. In this sense, when blowflies are attracted to an open wound, fly eggs are mainly found on the surface of the wound but not on the antlers (Azrolharith et al., 2022).

All the examined calliphorid larvae were molecularly identified as *L. caesar*, a facultative myiasis-causing agent. This species is widely distributed throughout Europe in both natural and synanthropic ecosystems (Szpila et al., 2013); their larvae mainly feed on animal carcasses, but also on living tissues as occurred in this study. Myiasis by *L. caesar* are mostly reported in captive wildlife (Gao et al., 2021), and the first case in free ranging wildlife was reported in a wild boar from Italy in 2021 (Pezzi et al., 2021).

Lucilia caesar is a common necrophagic species in northern Spain (Martínez-Sánchez et al., 2000; Moneo & Bordas, 2007). However, the absence of previous reports of pedicle myiasis in deer from this country suggests that this infestation could be an emergent disease. It has been reported that high temperature and humidity favour the development of *L. caesar* flies (Moneo & Bordas, 2007); therefore, this emergence may be favoured by global warming, which could increase fly populations and extend their activity periods. Moneo and Bordas (2007) stated that *L. caesar* is especially abundant in northern Spain during the hottest months. In this sense, Nielsen et al. (2010) suggested that the four cases of antler myiasis reported in Sweden were favoured by the existence of hot and humid weather conditions. Agreeing with these observations, all the cases described in the present study were detected in summer months (May–August). In addition, Moneo and Bordas (2007) also proved that *L. caesar* is more abundant in shaded habitats of forest areas, which are very abundant in the studied area.

It should be noted that another emerging myiasis caused by *Hypoderma actaeon*, considered specific for red deer, has been reported in roe deer from Central Spain in recent years (Panadero et al., 2017). Changes in the pattern of distribution of red deer and roe deer could have favoured the spreading of this myiasis between different host species.

It must be also pointed out that difficulties in tracking wildlife hinder the detection of pedicle myiasis in these animals; thus, most cases go unnoticed, leading to an underestimation of the incidence of this myiasis in free ranging ungulates. According to Obanda et al. (2013), the vulnerability to predation of the infested animals may explain why data on the incidence or epidemiology of cutaneous myiasis in free ranging wildlife are limited. However, in the present study, the collection and inspection of heads as part of a survey to determine the incidence of nasopharyngeal myiasis may have allowed the detection of pedicle myiasis cases that otherwise would have remained undiagnosed.

Physical examination revealed that all the animals presented an open cutaneous wound around the pedicles with deep ulcerations that, in some cases, were not external. In most affected bucks, massive larval infestations with extensive destruction of deep tissues and exposition of the frontal and parietal bones were found. In one animal, larvae had penetrated in the cranial cavity through a cranial suture, causing severe neurological signs such as blindness, deafness and ataxia. Similar lesions were described in four cases of severe pedicle myiasis caused by *L. illustris* in roe deer from Sweden, including circumferential skin ulcerations at the base of the antlers, exposition of the frontal bone and partial lysis of cranial sutures (Nielsen et al., 2010).

It is worth noting that larvae of *L. caesar* were also present in the nasal cavity of two bucks co-infected with *Cephenemyia stimulator*. These findings might indicate that the presence of *C. stimulator* could have attracted the larvae of *L. caesar* towards the nasopharyngeal cavity.

Three animals showed emaciation and behavioural alterations that recommended their euthanasia. Since necropsies did not reveal other diseases, it is very probable that this myiasis was the cause of these alterations. Traumatic myiasis in domestic animals causes important health injuries that need veterinary attention. However, in wild or extensively reared animals, with minor or absence of veterinary care, spontaneous recovery is rare and complications with secondary infections may result in septicaemia or toxaemia (Azrolharith et al., 2022). The severity of the cases described herein indicates that spontaneous recovery is unlikely, so further studies are needed to prevent and manage this myiasis in roe deer.

AUTHOR CONTRIBUTIONS

Néstor Martínez-Calabuig: Investigation; writing – original draft; methodology. **Rosario Panadero:** Conceptualization; writing – original draft; validation. **Susana Remesar:** Investigation; writing – review and editing; visualization. **David García-Dios:** Methodology. **Ana Saldaña:** Investigation. **Pablo Díaz:** Writing – review and editing; conceptualization. **Alberto Prieto:** Investigation. **Pablo Díez-Baños:** Funding acquisition; supervision. **Patrocinio Morrondo:** Funding acquisition; resources. **Ceferino M. López:** Formal analysis; visualization; writing – review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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