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First report of *Cephenemyia stimulator* (Diptera, Oestridae) parasitizing Roe deer (*Capreolus capreolus*) in Extremadura (Spain)

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Abstract

We describe the first two cases of parasitization by the dipterous parasite *Cephenemyia stimulator* in roe deer *Capreolus capreolus* (Linnaeus, 1758) in Extremadura. The first case was detected in an adult male from a private hunting estate (39º24' N, 5º32' W) in Berzocana (Cáceres, Spain). A total of 77 *C. stimulator* larvae were counted, of which 34 corresponded to instar II (L-II) and 43 to instar III (L-III), all of which were located in the upper airways. The second case was detected in another male roe deer from a local hunting estate (39º19' N, 5º22' W) in Logrosán (Cáceres, Spain), from which a total of 37 larvae (2 L-II and 35 L-III) were collected, all of which were located in the oronasal cavities. This paper not only provides the first report of this parasite in Extremadura, but also describes the southernmost occurrence of a potentially autochthonous infestation by *C. stimulator* in European roe deer. We recommend implementing surveillance measures in roe deer populations in Spain and creating strict biosecurity guidelines on translocations, especially regarding animals from affected areas. The presence of *C. stimulator* in Extremadura suggests it has adapted well to the ecological conditions of the southern Iberian Peninsula.
Keywords: *Cephenemyia stimulator*, roe deer, Extremadura, Spain, epidemiological surveillance, biosecurity.

Resumen

Se describen los dos primeros casos de parasitación por larvas del díptero *Cephenemyia stimulator* en corzos *Capreolus capreolus* (Linnaeus, 1758) de Extremadura. El primero de ellos se detectó en un corzo macho de un coto privado (39º24´ N, 5º32´ W) de Berzocana (Cáceres). El contaje de las larvas de *C. stimulator* recolectadas arrojó la cifra total de 77, de las cuales 34 correspondieron al estado II (L-II) y 43 al III (L-III), todas ellas localizadas en vías respiratorias altas. El segundo caso se diagnosticó en un corzo, igualmente macho, procedente de un coto local (39º19´ N, 5º22´ W) de Logrosán (Cáceres), en el cual se recogieron un total de 37 larvas (2 L-II y 35 L-III) todas ellas halladas en cavidades oronasales. Se trata de la primera cita de este parásito para Extremadura y la detección más meridional de la potencial infestación autóctona por *C. stimulator* en corzos europeos. Se recomienda la puesta en práctica de medidas de vigilancia epidemiológica en las poblaciones de corzos de nuestro país, y el diseño de pautas estrictas de bioseguridad en las translocaciones, especialmente de animales provenientes de zonas afectadas. La presencia del parásito en Extremadura sugiere una posible adaptación de *C. stimulator* a las condiciones ecológicas de la mitad sur de la península Ibérica.
Palabras clave: *Cephenemyia stimulator*, corzos, Extremadura, España, vigilancia epidemiológica, bioseguridad.

Introduction

Roe deer *Capreolus capreolus* (Linnaeus, 1758) have relatively recently been reincluded in the catalogue of game species of Extremadura following an increase in the few remaining populations in the Matallana Regional Hunting Reserve (Sierras de Los Ibores-Villuercas (Cáceres, Extremadura, Spain). Their reinclusion is linked to the rise in hunting activities over the last 20 years. Currently, roe deer populations range as far as the Almonte River, which is approximately 20 km from the city of Cáceres.

Cephenemyia stimulator Clark, 1815 (Diptera, Oestridae) is a dipterous parasite that causes cavitary myiasis in roe deer, for which it shows high specificity (Zumpt 1965). Ullrich (1936) was the first to highlight the veterinary importance of this parasite due to the clinical impact on its hosts. Other species of the genus have been described in wild cervids, such as *C. auribarbis* in red deer (*Cervus elaphus*) and fallow deer (*Dama dama*), *C. trompe* in reindeer (*Rangifer tarandus*), and *C. ulrichii* in moose (*Alces alces*) (Zumpt 1965, Nilssen *et al*. 2008).

Adult forms of *C. stimulator* are flies that look like bumblebees (*Bombus* spp.). Adults are 13-17 mm long, have a large head, large eyes, and small antennae; the body is covered with yellow-orange hairs that can sometimes be slightly reddish. Adults do not feed. Adults are good fliers, preferring open spaces. In some countries, such as Germany, they are active from June to late September, mainly during the morning and particularly between 11 to 12 a.m. Activity may vary between countries and even between regions. Males live for an average of 5 days, during which period they copulate repeatedly. Females live for about 16 days and once fertilized (holding up to 500 larvae in their abdomens) they look for roe deer in whose nostrils they deposit instar I (LI, 1-3 mm in length). The larvae migrate to the nasal cavities and paranasal sinuses, where they can remain dormant for months. After developing into L-II instars (3-13 mm long) and L-III instars (13-30 mm long) in the larynx and pharynx, they leave their hosts through the nostrils in late spring of the following year. They then pupate (16-20 mm long) in the soil and after a few weeks the new imagines emerge. This is therefore a univoltine life cycle (Zumpt 1965, Colwell 2001), as described for *C. trompe*.

Parasitization by *C. stimulator* in roe deer is of great clinical importance. Apart from the stress caused during larviposition, the presence of larvae in the upper airways causes sinusitis, sneezing, nasal discharge, coughing, dyspnoea, and swallowing problems. Asphyxia and bronchopneumonia associated with pneumotropic agents have been observed in the most severe cases (Cogley 1987). The severity of the process is closely related to the number of *C. stimulator* larvae present.

The process of parasitization by *C. stimulator* has been frequently observed in Central European countries, such as Hungary and the Czech Republic, where prevalences ranging from 34.6% to 44% have been recorded (Király & Egri 2007, Curlik *et al*. 2001). Similarly, seroepidemiological studies conducted in France have shown a 68% seropositivity rate (Maes & Boulard 2001).

The first studies on parasite fauna in roe deer in Extremadura revealed subcutaneous infestation by larvae of other Oestridae, such as *Hypoderma* spp. Nevertheless, the presence of *C. stimulator* was not detected (Navarrete *et al*. 1990). The first report of this parasite in roe deer in Spain was made by Notario & Castresana (2001). The roe deer came from France, presumably already infested, which were introduced into a hunting estate in the province of Ciudad Real in Spring 1997. According to the *Boletín del Corzo Español* (Spanish Roe Deer Newsletter; Anonymous 2009), in 2004-05 the parasite was detected in the region of Asturias (Valdés county, Spain) in roe deer from the northern Pyrenees. There have also been reports of new cases in other areas of Asturias, as well as in Lugo (Los Ancares), León (El Bierzo, Babia, Omaña, Laciana, etc.), Palencia, and Burgos, suggesting that its appearance is due to the reintroduction of roe deer from areas where the parasite is endemic.

Regardless of the source of the parasite, however remote, or whether this situation is a consequence of translocations undertaken without biosecurity measures, *C. stimulator* is currently suspected of having become established in roe deer populations in the northern and central Iberian Peninsula. The consequences could be severe for roe deer due to the risk to their health and the difficulties involved in controlling the parasite.

This study describes the first two cases of parasitism by *C. stimulator* in the southern half of the Iberian Peninsula (Cáceres) in native roe deer populations, and is particularly motivated by the fact that to date there are no records of authorized translocations being conducted in this area.

Materials and Methods

In May 2012, two male roe deer captured in different hunting estates were sent to the Unit of Parasitology, Faculty of Veterinary Medicine, Cáceres. The first deer was captured at 39º24′ N, 5º32’ W (municipality of Berzocana) and the second at 39º19′ N, 5º22′ W (Logrosán area), both in the province of Cáceres (Fig. 1).

A formal necropsy was performed in the first roe deer and conventional parasitological diagnosis tests (coprology, search, collection and identification of

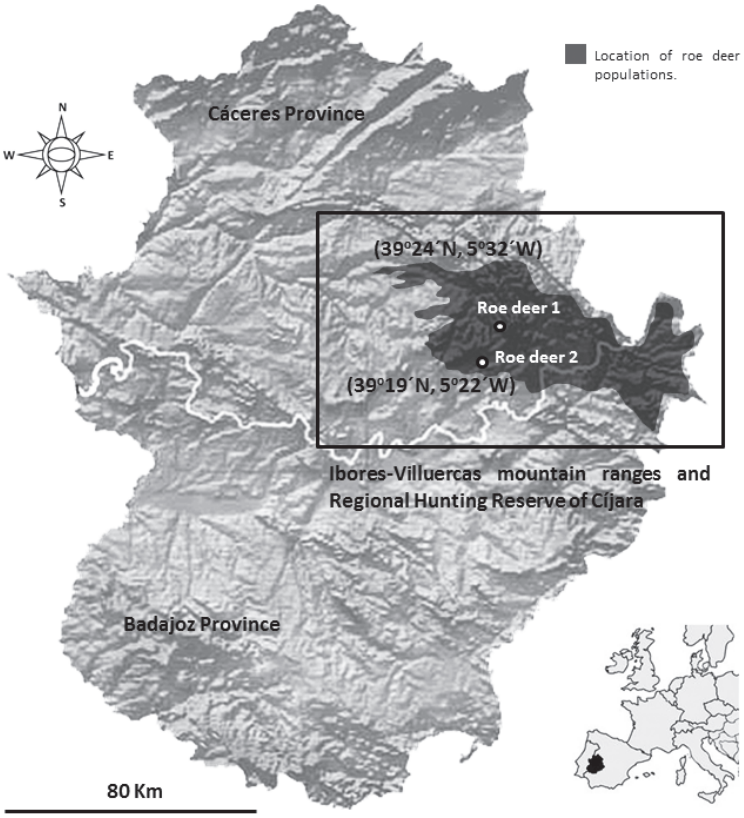

Figure 1. Location of cases of infestation by *C. stimulator* in roe deer from Extremadura (Spain). Case 1: Adult, male, captured 14th May 2012 in a private estate in Berzocana (Cáceres). Case 2: Adult, male, captured 27th May 2012 in a public estate in Logrosán (Cáceres).

endo- and ectoparasites, etc.) and bacteriological tests (culture and identification using the BD Phoenix System, [BD, Franklin Lakes, USA]) were conducted. In both cases the head was sectioned longitudinally from the dorsal angle of the occiput to the rostral border of the nasal bone, allowing a full examination of nasal and pharyngeal cavities and paranasal sinuses. All larvae found were collected, washed in saline solution and placed in ethanol (70% v/v) until they were identified according to the detailed description given by Zumpt (1965).

Results

Firstly, it should be noted that all larvae collected from both animals (n = 114) were identified as *C. stimulator*. In both cases, instars II and III were identified on the basis of their morphological and morphometric characteristics. Instars II were longer than 13 mm, with an unarmed tenth segment, and between 4 and 6 rows of spines on the remaining segments. Instars III were longer than 30 mm, presenting a characteristic rear reniform peritreme (Fig. 2). No imagine emerged from the incubated pupae.

This is the first recorded case in Extremadura and describes the southernmost occurrence of potentially autochthonous infection by this parasite in the Iberian Peninsula, and therefore in Europe. We now describe further points of interest for each of the cases.

Case no. 1: the animal presented with general malaise, weakness, poor body condition, unsightly hair, and with velvet still present on the antlers. The animal also exhibited apathy and low vitality before dying. Anatomopathological findings revealed abundant greenish mucus in the nostrils, sinusitis, laryngitis and pharyngitis; pneumonia was also suspected with patent pulmonary hepatisation, congestion, and the presence of abundant exudate. Bacteriological analyses revealed the presence of *Pasteurella* spp. in lungs. Nymphs of the tick species *Rhipicephalus bursa* at different body locations were shown by parasitological analyses, as well as the nematode *Gongylonema pulchrum* in esophageal submucosa and the protozoan *Sarcocystis* spp. in cardiac muscle. Finally, 77 *C. stimulator* larvae were collected, of which 34 were L-II and 43 were L-III (Fig. 2). The larvae L-II were mainly located in the pharynx and the larvae L-III in the oropharynx and nasal turbinates. This location was due to their need to exit the host's body, since their natural location is always the retropharyngeal cavity. There were no further

findings of interest, although it is perhaps worth noting the presence of some gastrointestinal and lung nematodes, such as *Teladorsagia circumcincta*, *Spiculopteragia asymmetrica*, *Nematodirus filicollis*, and *Muellerius capillaris* in small numbers (<100 eggs/gram of faeces).

Case no. 2: external examination of the animal only revealed unsightly hair. However, visible signs of laryngitis, pharyngitis, and sinusitis were seen macroscopically. Ticks (adult *Rhipicephalus bursa*) and 37 *C. stimulator* larvae (2 L-II and 35 L-III) were present. The larvae were mainly located in the retropharyngeal and nasal cavities in readiness to exit the host.

Discussion

This is the first report of *C. stimulator* in roe deer in Extremadura. It confirms our suspicions that *C. stimulator* is the oestrid parasite often described by individuals involved in roe deer hunting and management who blame *C. stimulator* for the

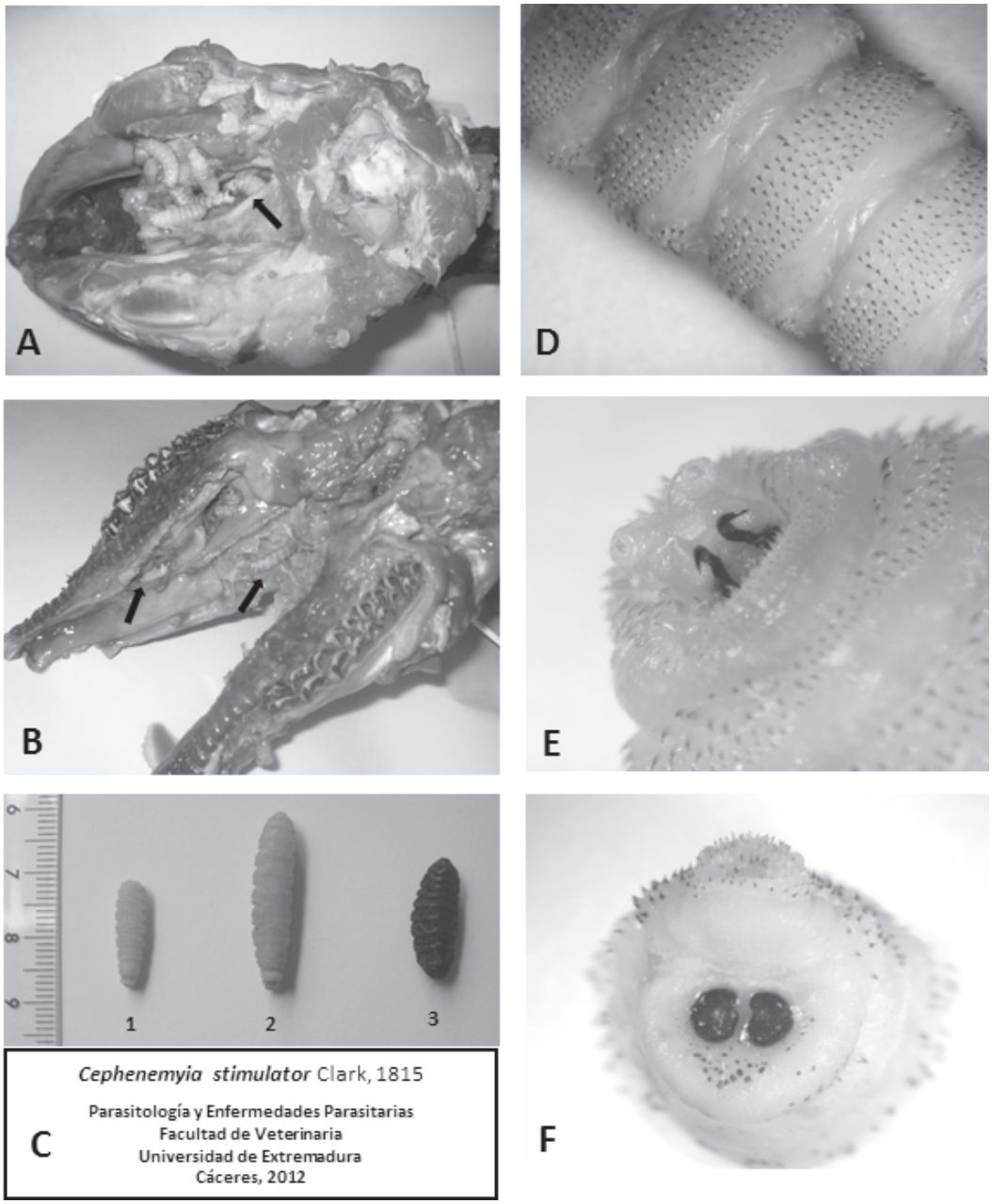

Figure 2. Pharyngeal (A) and oronasal (B) location of *C. stimulator* larvae. Larvae II (C1), larvae III (C2), and puparium (C3). Identifying structures of *C. stimulator*: 5-8 rows of dorsal spines (D); cephaloskeleton (E); reniform peritremes (posterior view) (F).

decline in roe deer populations in the areas referred to above.

It is difficult to determine the origin of this parasite in roe deer in Extremadura. Possible reasons for its presence in the study area include its introduction following roe deer translocations (unconfirmed in Extremadura), its expansion from nearby potentially endemic areas due to host movement, and increased environmental favourability caused by global warming involving warmer autumns and springs that could extend the period of activity of imagines and their distribution range (Taylor 2009). Whatever the case, these would all be difficult to confirm.

Our findings are consistent with those published by other authors (Rivosecchi *et al*. 1978, Maes & Boulard 2001, Vaca 2001, Király & Egri 2004) who consider *C. stimulator* to be the oestrid parasite of roe deer. However, there have been reports of accidental infestations by *Pharyngomyia picta* (Sugar 1974) in roe deer and also by *C. stimulator* in red deer (Király & Egri 2004).

Several authors (Király & Egri 2004, Sugar *et al*. 2004) agree that young animals are more vulnerable to *C. stimulator* parasitization due to lower immune competence and their reduced ability to evade fly attacks. Males form the next most vulnerable group with no difference between age groups. The susceptibility of males could be related to the effort involved in guarding their territories, social stress, the loss of energy involved in mating, and so on. These behaviour-associated constraints may lead to an immunocompromised state leaving them susceptible not only to this parasite but also to other diseases. This may translate into poor trophy quality. For example, the animal described in case 1 was weak, had a very poor overall appearance, and had velvet remaining on its antlers.

The parasitic loads of *C. stimulator* observed can be considered high, as they were much higher than the average infestation levels described by Barth *et al*. (1976) in roe deer in Germany (3-11 L/animal), by Curlik *et al*. (2001) in the Czech Republic (13 L/animal), or by Király & Egri (2004) in Hungary (8.8 L/animal). This high load was the most probable cause of the evident deterioration observed in case 1, in which the small loads of gastrointestinal and pulmonary helminths could not in themselves explain the animal's poor condition of health.

Regarding the development of the larval stages, in approximately the 15 days between the capture of the first deer (14 May 2012) and the second (27 May 2012), the L-III became predominate indicating the rapid development of larvae in spring. This is in line with Dudzinski (1970), who stated that L-II can be present between April and July, and L-III between April and August in Central European countries. We were unable to create the conditions needed for the emergence of *C. stimulator* imagines in our study. This was probably due to the relatively low temperatures and levels of humidity in the pupation incubators. These were much lower than the conditions (14.8ºC [7.5-21]; 64.4%RH [37-91]) proposed for *Hypoderma diana* by Jorrín & Gasca (1992) and presumably lower than those required and found in their ecological niches (Vaca 2001, Király & Egri 2004, Nilssen *et al*. 2008).

In conclusion, the massive presence of the larvae observed in both animals could have been the cause of their weakness, poor body condition, and state of immunosuppression similar to that observed in other domestic and wild species due to parasitism by Oestridae (Alcaide *et al.* 2003); this level of infestation may therefore make them susceptible to diseases caused by output pathogens (latent, saprophytes, etc.), sometimes with fatal consequences. Less intense infestations may cause weakness, reproductive wastage, and decreased trophy quality. In any case, and given the high levels of parasitism observed, further studies are needed on the role of *C. stimulator* in relation to roe deer population decreases in certain areas of Spain (Anonymous, 2009) and also in Extremadura. Close cooperation should be established between hunters, managers, authorities, and researchers to obtain the basic epidemiological information needed to develop future programmes to control this and other roe deer diseases.

We strongly recommend that translocations should be controlled and conducted under strict biosecurity measures. At the very least, we urge the use of chemoprophylaxis using macrocyclic lactones (ivermectin and milbemycins) to control these parasites and others sensitive to these drugs. It is a matter of some urgency to prevent the spread of pathogens and diseases that may have serious consequences on wildlife, livestock, and human populations.

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