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ORIGINAL ARTICLE

Report of *Oslerus rostratus* (Strongylida: Filaroididae) in cats from the Canary Islands, Spain

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ABSTRACT. Metastrongylid species infecting wild and domestic cats worldwide are increasingly being reported. Between 2017 and 2019, a total of 202 faecal samples of domestic cats from the island of Tenerife (Canary Islands, Spain) were analysed by microscopy and molecular techniques. Morphological analyses showed that 8.91% (18/202) of the faecal samples presented first stage larvae (L1) of metastrongylid species. Total DNA was isolated and tested by PCR targeting a 508 bp fragment of the ITS-2 gene. The nucleotide sequences obtained showed high homology (100%) with the species *Oslerus rostratus*. This work contributes to the knowledge of the wide distribution of *O. rostratus* worldwide, being Tenerife (Canary Islands, Spain), close to the African continent, the new geographic location for this metastrongylid species. Further molecular studies involving new geographic areas from the island of Tenerife, as well as neighbouring islands, are needed to provide relevant insight and better understand the epidemiology of *O. rostratus* and other metastrongylid species in wild and domestic cats from the Canary Islands.

Keywords: Oslerus rostratus, metastrongylids, lungworm, domestic cat, Canary Islands, Spain.

INTRODUCTION

Metastrongylid species (Nematoda: Metastrongyloidea) have been frequently reported to infect felids worldwide. These parasites are recognised as important etiological agents in the pathology of the cardio-pulmonary system of felids and have gained the attention of the veterinary community (Traversa *et al.*, 2010; Brianti *et al.*, 2014). Infections by metastrongylid nematodes in felids can be asymptomatic or may show a variety of clinical signs and symptoms depending on the age and immune status of the host, the parasite species, and the parasitic burden. Besides, depending on the degree of infection, it can be fatal (Di Cesare *et al.*, 2011; Traversa & Di Cesare, 2013; Pennisi *et al.*, 2015).

The signs of infection by metastrongylids species are similar to those of other feline respiratory diseases such as feline asthma, and allergic bronchitis, among others (Foster & Martin, 2011). In addition, the first-stage larvae (L1) of the different metastrongylid species found in the faeces of infected hosts have similar morphometric characteristics (Traversa & Di Cesare, 2013), which makes their identification a challenge and, therefore, it should be considered by veterinarians in order to make a correct diagnosis, highlighting the usefulness of molecular tools, especially in epidemiological surveys on lungworm infections in both domestic and wild animals (Otranto *et al.*, 2013; Brianti *et al.*, 2014; Penagos-Tabares *et al.*, 2018).

The metastrongyloid *Aelurostrongylus abstrusus* (Strongylida: Filaroididae) is the most common and widespread nematode reported to infect the domestic cat, followed by the trichurid *Eucoleus aerophilus* (syn. *Capillaria aerophila*) (Anderson, 2000; Traversa *et al.*, 2010; Di Cesare *et al.*, 2015; Traversa and Di Cesare, 2016; Giannelli *et al.*, 2017). Besides, other metastrongylid nematode species have been cited to affect cats, namely *Angiostrongylus vasorum* (Traversa and Guglielmini, 2008), *Oslerus rostratus* (Brianti *et al.*, 2014), *Gurtlia paralysans* (Moroni *et al.*, 2012), and *Troglostrongylus* species (Brianti *et al.*, 2012), among others.

In Europe, these nematodes have mainly been studied in wild and domestic cats, *Felis silvestris* and *Felis catus*, respectively (Traversa & Di Cesare, 2016). In the Canary Islands (Spain), an archipelago composed of eight islands and islets situated close to the NW side of Africa, studies related to the distribution and prevalence of metastrongylid species in cats are scarce. An anatomopathological study on domestic dead cats, carried out in 1992, was the first to provide data about the presence of *A. abstrusus* in this archipelago (Valladares *et al.*, 1992). Also, an epidemiological survey carried out on the island of Gran Canaria revealed an overall prevalence of 10.4% of *A. abstrusus* in feral *F. catus* (Rodríguez-Ponce *et al.*, 2016). Recently, *G. paralysans* was first reported in the Canary Islands parasitising the eye of a domestic cat (Udiz-Rodríguez *et al.*, 2018). Considering data is currently scarce, the present study aimed to detect the presence, as well as the identity and prevalence, of the metastrongylid

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lungworm species that could be affecting domestic cats from Tenerife (Canary Islands, Spain).

MATERIAL AND METHODS

Between 2017 and 2019, a total of 202 faecal samples of domestic cats from 1 to 3 years old from Tenerife, Canary Islands (Spain), were sent for routinely parasitological analyses to the Finca España Laboratory, a private centre that performs a parasitic diagnosis service for veterinary clinics. The remaining sample, previously diagnosed, was stored frozen at -20ºC. Later, this collection was analysed for the search for metastrongylid larvae. Due to the current legislation (R.D. 53/2013) and considering the origin of these samples, previously analysed at a laboratory centre for routine analyses, no ethical approval was required.

After thawing, all faecal samples were concentrated by using a modification of Richie's formaldehyde-ether method, in which the formaldehyde-ether was replaced by ethyl acetate (Young *et al.*, 1979) and analysed to screen the samples for metastrongylid L1 larvae. It was not possible to find out any information about the previous provenance or travel histories of the animals and their outdoor activities. Larval body length, the position of the oral opening and tail morphology of the larvae found in the faecal samples were the main features considered in the morphological identification. Data obtained were compared with the descriptions reported elsewhere (Traversa and Di Cesare, 2013; 2016), commonly used for *A. abstrusus*, *Troglostrongylus brevior* and *O. rostratus* differentiation. Digital images and measurements in µm were taken using the optical microscope Leica DM2500 and the Leica LAS AF 4.12 software, respectively.

For the molecular study, genomic DNA was extracted using the faecal concentrate which was diluted in 250 µl of a solution containing 30 mM Tris-HCL (pH 8.0), 10 mM EDTA and 0.4% SDS. Then, 3 µl of proteinase K (20 mg ml $^{-1}$) was added to the samples and incubated at 56 ºC overnight. After having inactivated the proteinase K, DNA extraction continued following the instructions of the method used by Lopez *et al.* (2015). The quantity and quality of the extracted DNA were determined with the spectrophotometer Nanodrop ND-1000 (Thermo Fisher Scientific, Wilmington, DE, USA). DNA was stored at -20 ºC until further processing. A fragment of the internal transcribed spacer 2 (ITS-2) was amplified using the primers NC1 (5'-ACGTCTGGTTCAGGGTTGTT-3') and NC2 (5'-TTAGTTTCTTTTCCTCCGCT-3') as previously described by Gasser *et al.* (1993). Approximately 20-50 ng of genomic DNA were added to each PCR. PCR reactions were performed in a total volume of 25 µl, including 10× buffer Mg^{2+} free (Bioline, London), 2.5 µl of each dNTP (10 mM), 1 µl of each primer (12.5 ng/ml), 0.125 µl of Biotaq polymerase (5 U/ml) (Bioline, London), 0.75 mM $MgCl_2$, 2 µl of DNA template and water. The ITS-2 fragment was amplified using the following cycling conditions: 94 °C for 2 min (first polymerase activation and denaturation), followed by 35 cycles of 94 °C for 1 min (denaturation), 58 °C for 1 min (annealing), and 72 °C for 1 min (extension), with a final extra extension step at 72 °C for 5 min. All PCR products were resolved on 1.5% agarose gels. The amplicons were sequenced in both directions in SEGAI (Universidad de La Laguna sequencing services, Spain) and Macrogen Inc. (Madrid, Spain). The obtained sequences were edited with the MEGA X program (Kumar *et al.*, 2018) and subsequently aligned with the ClustalW program included in MEGA X. To elucidate any homologies or similarities previously published in GenBank, a BLAST search was carried out. The molecular identification, carried out in MEGA X, was achieved by phylogenetic analysis through the Neighbor-Joining distance method (Saitou & Nei, 1987) with at least 1000 bootstrap replications. *Toxocara cati* was used as the outgroup. Nucleotide sequence data reported in this publication is available in the GenBank database under the accession numbers: MW263070, MW263071 and MW263072.

RESULTS

First stage larvae (L1) of metastrongylid species were detected by microscopical analysis in 18 out of 202 (8.91%) faecal samples from domestic cats. A total of 50 first stage L1 larvae were measured, obtaining body length values ranging from 333 to 390 µm, with 361.1 as the mean value and 17 of standard deviation. When compared with the larval length reference of metastrongylid species, the measurements values obtained were within the interval range reported for *A. abstrusus* (360-415 µm), *T. brevior* (300-357 µm) and *O. rostratus* (335-412 µm). These measured larvae were characterised by a rounded head with a cylindrical buccal capsule and a central oral opening surrounded by a cuticular ring with dorsal and ventral prominences. The tail was slightly undulated, with a deep ventral and a shallow dorsal one ending with a minute spine, matching this morphology description with the reported for the species *O. rostratus* (figure 1).

Since using the morphological and morphometric diagnosis of the L1 larvae of metastrongylid species could cause misidentification, molecular analyses based on the ITS-2 gene were carried out. Eventually, only three out of 18 samples were successfully sequenced. The three nucleotide sequences obtained, isolate 017 (434 bp), isolate 976 (468 bp) and isolate 694 (431 bp), displayed 99-100% homology with *O. rostratus* sequence of *F. catus* from Sardinia, Italy (GenBank: KM009118) and with other *O. rostratus* sequence of *F. silvestris* from Hesse, Germany (GenBank: KP987220). Furthermore, phylogenetic analysis based on 454 bp of the alignment confirmed these results, since the sequences from domestic cats obtained in this study were grouped into the *O. rostratus* clade with high bootstrap value (100) (figure 2).

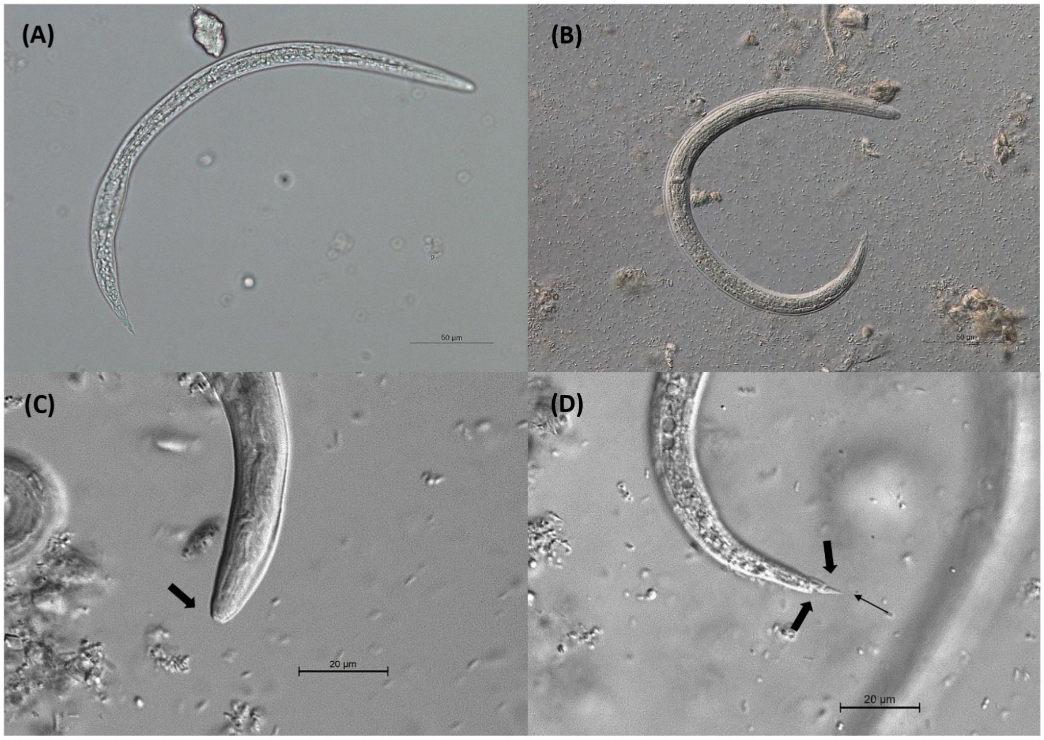

Figure 1. First-stage larvae (L1) of *Oslerus rostratus* (A, B) recovered from cat fecal samples; (C) magnification of the anterior end, showing a rounded head with a central oral opening surrounded by a cuticular ring with dorsal and ventral prominences (bold arrow); (D) magnification of the tail, showing a deeper notch on the ventral side and a shallower one on the dorsal side (bold arrows). At the proximal edge of the dorsal notch there is a minute cuticular spine (light arrow).

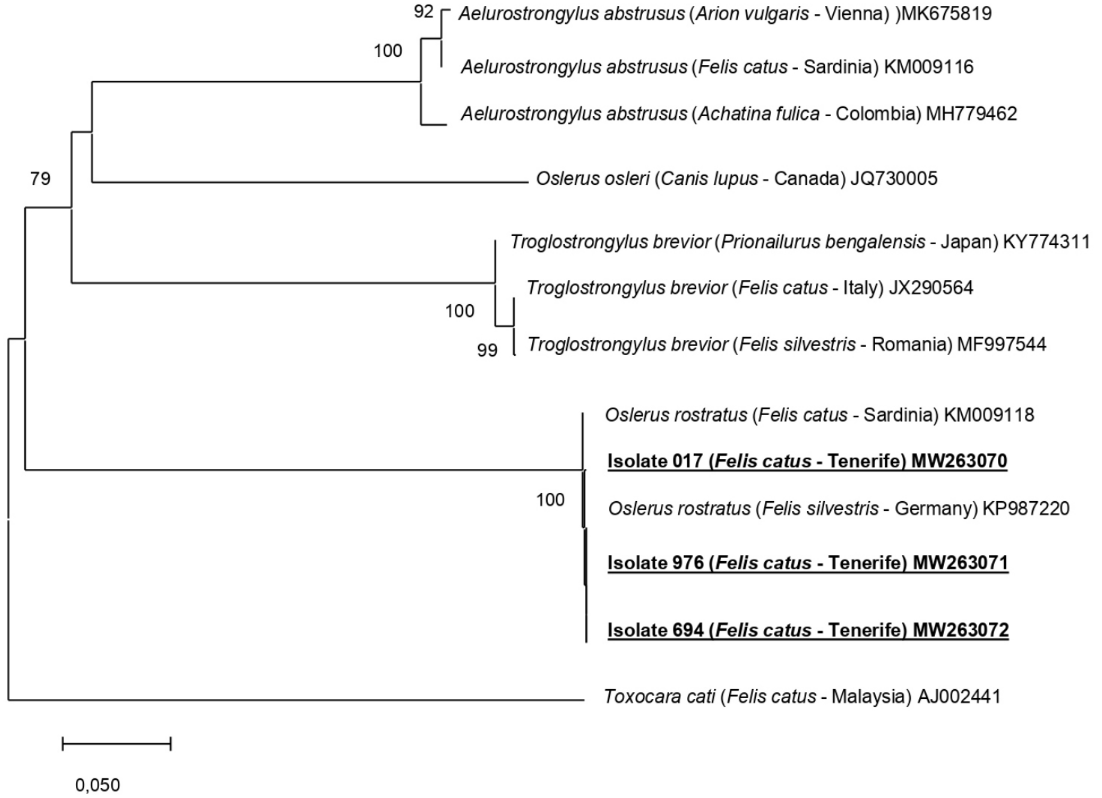

Figure 2. Phylogenetic analysis using the Neighbor-Joining method with p-distance and 1000 bootstrap replications based on a 454 bp fragment of the internal transcribed spacer 2 (ITS-2). New sequences obtained in this study are typed in bold, underlined text. *Toxocara cati* was used as the outgroup.

DISCUSSION

The results obtained in this study contribute to deepening the knowledge about feline lungworms distribution. *Oslerus rostratus*, after its first description as *Anafilaroides rostratus* in cats from Jerusalem (Gerichter, 1949), was reported in cats from Sri Lanka island (Seneviratna, 1958) and Hawaii island (Ash, 1962), as well as in bobcats from Virginia and Georgia (USA) (Klewer, 1958; Watson *et al.*, 1981). In Europe, this nematode has been reported in domestic cats from mainland Spain (Juste *et al.*, 1992), in feral cats from Majorca and domestic cats from Ibiza islands (Spain) (Millan & Casanova, 2009; Jefferies *et al.*, 2010), in a stray cat from Sicilia and a domestic cat from Sardinia islands (Italy) (Brianti *et al.*, 2014; Varcasia *et al.*, 2015) and domestic cats from Hungary (Kiszely *et al.*, 2019). Recently, *Oslerus sp.* has been found in South America, more specifically in Brazil and Chile, parasitising two felid species, the jaguarundi (*Puma yagouaroundi*) and the guignas (*Leopardus guigna*), respectively (Corrêa *et al.*, 2019; Acuña-Olea *et al.*, 2020). Therefore, our study provides new data on the distribution of *O. rostratus*, being the seventh report on islands.

With regard to the prevalence, in Europe, a study carried out by Giannelli *et al.* (2017) showed that 10.6% (n= 210/1990) of the sampled domestic cats were infected by lungworms. The prevalence of lungworm infection reported for Spain was 6.5% (n= 13/200), being similar to the prevalence obtained in our study, 8.91% (n= 18/202). In the same study, 3.8% (n= 8/210) of the lungworm infections in domestic cats were caused by the species *O. rostratus*. However, the higher prevalence was obtained for *O. rostratus* in other regions, such as Sri Lanka, Virginia (USA) and Majorca island (Spain), where 60%, 96% and 24% of prevalence were reported, respectively (Seneviratna, 1958; Klewer, 1958; Watson *et al.*, 1981; Millán & Casanova, 2009). Despite this, the reports of this nematode in domestic cats are usually regarded as singles cases (Gerichter, 1949; Juste *et al.*, 1992; Brianti *et al.*, 2014; Varcasia *et al.*, 2015).

According to the life cycle of *O. rostratus*, it is similar to other metastrongylid species, with a wide range of mollusc species acting as intermediate hosts (Seneviratna, 1959). Furthermore, species of lizards, frogs, birds, and small mammals can act as paratenic hosts, thus contributing to the dispersion and transmission of metastrongylids species among this fauna, including cats (Bowman, 2000, 2002). In the Canary Islands, there are numerous species of terrestrial gastropods, some of them endemic, as well as lizards, frogs, birds and small mammals (Izquierdo *et al.*, 2004). In this sense, some introduced metastrongylid species, such as *Angiostrongylus cantonensis*, have been successfully adapted to the Canaries habitat since it was previously found in rats (*Rattus rattus*) and terrestrial snails from Tenerife (Foronda *et al.*, 2010; Martin-Alonso *et al.*, 2015). In addition, an epidemiological study carried out in this archipelago reported the presence of A. *cantonensis*, *A. vasorum* and *A. abstrusus* in terrestrial native slug and snails species collected in the islands of Tenerife, Gran Canaria, El Hierro, Lanzarote, La Palma and Fuerteventura (Segeritz *et al.*, 2021). More studies would be necessary to confirm if the life cycle of *O. rostratus* is well established in the island of Tenerife and if the free ranging domestic cats could have made them available to infect potential intermediate and paratenic hosts.

On the other hand, veterinarians from the island of Tenerife have reported the presence of *A. abstrusus* larvae in the faeces of domestic cats (author's pers. obs.). However, the larvae diagnosis in those clinical cases has been made only by morphological techniques, so *O. rostratus* could be more prevalent in the Canary Islands than currently thought, being misdiagnosed as *A. abstrusus* due to the overlapping morphological features and individual variations of the metastrongylid L1 larvae (Traversa & Di Cesare, 2013). Furthermore, if both metastrongylid species *A. abstrusus* and *O. rostratus* have been detected in the Canary Islands, coinfections by these two metastrongylid species in a domestic cat could be occuring, as previously reported by other studies carried out in Spain, Sicily, and Hungary (Juste *et al.*, 1992; Brianti *et al.*, 2014; Kiszely *et al.*, 2019; Gianelli *et al.*, 2017). Even though molecular analyses from this study could not confirm the presence of coinfection, they should not be discarded.

The current climate change together with ecological factors, international trade, travel and migration, and animals transport are factors than can influence the establishment, maintenance, and transmission of metastrongylid species in previously unreported areas (Patz *et al.*, 2000; Traversa *et al.*, 2010; Brianti *et al.*, 2014; Otranto, 2015; Traversa & Di Cesare, 2016). An example is the Canarian Archipelago, where a total of two metastrongylid species have been reported infecting cats: *A. abstrusus* in feral cats from Gran Canaria (Rodríguez-Ponce *et al.*, 2016) and *G. paralysans* in domestic cat from Tenerife (Udiz-Rodríguez *et al.*, 2018). Therefore, the report of *O. rostratus* in our study constitutes the third report of a metastrongylid species parasitising cats in the Canary Islands, Spain.

This work contributes to the knowledge of the wide distribution of *O. rostratus,* previously reported in Europe, Asia and America, being Tenerife (Canary Islands, Spain), the new geographic location for this metastrongylid species. Further studies involving neighbouring islands of the Canary Islands will provide relevant insight to better understand the epidemiology of *O. rostratus* and other metastrongylid species in wild and domestic cats from the Canary Islands.

COMPETING INTERESTS STATEMENT

The authors declare that they have no competing interests.

ETHICS STATEMENT

Due to the current legislation (R.D. 53/2013) and considering the origin of these samples, previously analysed at a laboratory centre for routine analyses, no ethical approval was required.

AUTHOR CONTRIBUTIONS

KGL: methodology, data analysis and interpretation, writing original draft, review and editing. MVS and SP provided resources, review and editing of the manuscript. BV and PF conception and design of the study, resources, validation, review and editing the manuscript, visualisation, and supervision. All authors read and approved the final manuscript version to be submitted.

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