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Short Communication

Resolution of canine ocular thelaziosis in avermectin-sensitive Border Collies from Spain

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ABSTRACT

Ocular thelaziosis by *Thelazia callipaeda* is an emerging disease that affects primarily dogs, but also cats, foxes and other wild carnivores, as well as humans. Three clinical cases of unilateral conjunctivitis caused by *Thelazia* nematodes were detected in Border Collie, a dog breed intolerant to the macrocyclic lactones. Animals came from southwestern Spain, on the border with Portugal. Eight worms were collected and identified molecularly as *T. callipaeda* by amplification and sequencing of partial cytochrome *c* oxidase subunit 1 gene. Oral treatment with mebendazole 20 mg/kg (Telmin®) was effective in curing the infection.

Keywords: Thelazia callipaeda, *cox*1, haplotype 1, Border Collie, mebendazole, Spain

1. Introduction

Thelazia callipaeda (Spirurida, Thelaziidae) eyeworms infect the orbital cavities and surrounding tissues of carnivores and humans, causing from mild (i.e., conjunctivitis, epiphora, ocular discharge) to severe (i.e., keratitis, corneal ulcers) ocular

condition (Anderson, 2000). This nematode is transmitted by the fruit fly, *Phortica variegata* (Diptera, Drosophilidae, Steganinae), which releases infective third-stage larvae in the conjunctival sacs while feeding on the lacrimal secretions of a receptive host.

Thelaziosis, apart from dogs (Malacrida et al., 2008) and humans (Shen et al., 2006; Otranto and Dutto, 2008; Fuentes et al., 2012), may also affect wild carnivores (Otranto et al., 2009; Calero-Bernal et al., 2013). The distribution of the infection comprises several Asian and European countries (Otranto and Dutto, 2008) and its spreading in previous non endemic areas is related with the distribution of its vector (Otranto et al., 2006). Since the first case of infection in dogs and cats from Spain (Miró et al., 2011), new cases have been reported in the Iberian Peninsula (Fig. 1). All the *T. callipaeda* isolates for which sequences of partial cytochrome *c* oxidase subunit 1 (*cox*1) are available belong to the haplotype 1 (h1), suggesting a high degree of nematode-host affiliations for this haplotype (Otranto et al., 2005).

Control of canine thelaziosis is currently based on the removal of nematodes directly from the eyes of affected dogs or relies on chemotherapy by local instillation of antiparasitic drugs like imidacloprid-moxidectin formulation (Bianciardi and Otranto, 2005), moxidectin (Lia et al., 2004), or oral administration of milbemycin oxime (Ferroglio et al., 2008). In addition, the commercial formulation of milbemycin oxime at the minimal dose of 0.5 mg/kg and 2 mg/kg, showed a high efficacy in curing *T. callipaeda* infections in dogs and cats, respectively (Motta et al., 2012). Nevertheless, there is a problem in cases of infection in Collies and related breeds, because their sensitivity to the macrocyclic lactones, due to a mutation of the MDR-1 gene. Therefore, in Collies, a wider margin of safety has been recorded for moxidectin than

for ivermectin or milbemycin by formulation at 30, 60, or 90 µg/kg (Paul et al., 2000). In this study we report for the first time three cases of canine ocular thelaziosis in Border Collies from southwestern Spain and their treatment.

2. Material and methods

2.1. Case report

The three Border Collies included in this case series lived in the same farm located in the municipality of San Vicente de Alcántara, southwestern Spain (39º19´14´´N, 7º1´41´´W) on the Portugal-Spain border (Fig. 1). The farm comprised an area of 700 ha, where cattle, sheep and pigs were raised in free-ranging conditions. The area where dogs lived was featured by a semi-desert Mediterranean ecosystem (average annual rainfall of 700 mm and average annual temperature of 18ºC) composed by herbs, bushes and trees, mainly oaks (i.e., *Quercus rotundifolia* and *Q. suber*) and shrubs (i.e., *Cistus ladanifer* and *Cytisus scoparius*).

The shepherd referred that dogs repeatedly stopped their march to grub their eyes with the forelimbs. The ocular inspection revealed moderate conjunctivitis and nematodes were found into the conjunctival sacs. Previous instillation of ophthalmic anesthetic with naphazoline and tetracaine (Colircusí Anestésico 0.5%, Alcon Cusí®, Masnou, Barcelona, Spain), eight adult nematodes were collected with swabs from the conjunctival sacs by flushing with saline solution (0.9% NaCl) and stored in 70% ethanol. The dogs, weighing 21.7, 23.6 and 24.5 kg, were orally treated with mebendazole 20 mg/kg (Telmin® suspensión, Esteve, Barcelona, Spain) for three

consecutive days. Animals were checked weekly in order to identify the possible presence of nematodes; no relapses have been detected until date. None of other dogs of different breeds (three Spanish Mastiff and a Golden Retriever), coexisting in the same farm, were affected.

2.2. Morphological and molecular identification

Nematodes specimens were identified using morphological keys (Skrjabin et al., 1967; Otranto et al., 2004). Worms were also subjected to specific PCR amplification and sequencing of a partial (689 bp) *cox*1 gene fragment (Otranto et al., 2005) and the sequences were compared with those available in the GenBank database by Basic Local Alignment Search Tool (BLAST –http://blast.ncbi.nlm.nih.gov/Blast.cgi).

3. Results and discussion

Eight adult worms were retrieved (i.e., 2 males and 6 gravid females), of these, 1 male nematode was extracted from the right eye of the first dog, 1 male and 1 female nematode from the left eye of the second one, and the remaining 5 female worms from the right eye of the third Border Collie (Fig. 1). All of them were morphologically and molecularly identified as *T. callipaeda*. Molecular identification by BLAST analysis of the *cox*1 sequences herein analyzed showed a 100% nucleotide identity with a sequence of *T. callipaeda* h1 available in GenBank database (i.e., AM042549).

Ocular thelaziosis by *T. callipaeda* have been previously detected in canids and felids in the Iberian Peninsula (Fig. 1), in Italy, France and Switzerland (Otranto et al.,

2003; Dorchies et al., 2007; Malacrida et al., 2008). The retrieval of three infected dogs at latitudes southernmost of the original focus in La Vera region (Miró et al., 2011) may suggest an expansion in the vector distribution or even the introduction of infected dogs in areas where the vector was already present. Indeed, even if this has not been confirmed yet, such territories fall outside the geoclimatic provisional model for the distribution of the arthropod vector (Otranto et al., 2006), suggesting that future studies on the habitat suitability for *P. variegata* fruit fly would provide valuable information on the risk areas for *T. callipaeda* transmission in Europe. Also, *cox*1 sequences confirmed the presence of *T. callipaeda* h1 at the border of Alentejo region of Portugal, as well as in the Iberian Peninsula (Miró et al., 2011; Soares et al., 2013; Calero-Bernal et al., 2013) and the rest of Europe (Otranto et al., 2009).

This is the first report of *T. callipaeda* in Border Collies. In addition, four dogs of Spanish Mastiff and Golden Retriever breeds cohabiting in the same farm were not parasitized. This might be due to the fact that Border Collies were used for shepherding in different locations; whereas the other breeds only for guarding around the facilities (i.e., no movements or travels were declared).

Resolution of the infection was achieved by a pharmacological treatment with mebendazole 20 mg/kg as an alternative to avermectins, even if security margins of moxidectin were proposed (Paul et al., 2000). This study adds another option in the list of drugs that have been proven effective against *T. callipaeda* in dogs, which include ivermectin 1% subcutaneous (200 µg/kg) (Rossi and Peruccio, 1989), instillation of moxidectin 1% (6 mg) (Lia et al., 2004), topical formulation of imidacloprid 10% and moxidectin 2.5% (Bianciardi and Otranto, 2005) and orally milbemycin oxime 0.5 mg/kg (Ferroglio et al., 2008). Noteworthy, it is important to remove adult worms and

administrate antiparasitic drugs to kill the remaining larvae and to prevent possible reactivation of the infection. Finally, knowledge of this parasitosis is relevant for veterinary and medical clinicians due to its emergent character, which is most likely associated to *P. variegata* fruit flies. Future research should be focused to enhance knowledge on reservoir species and vectors in the study area.

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238 **Figure captions**

239

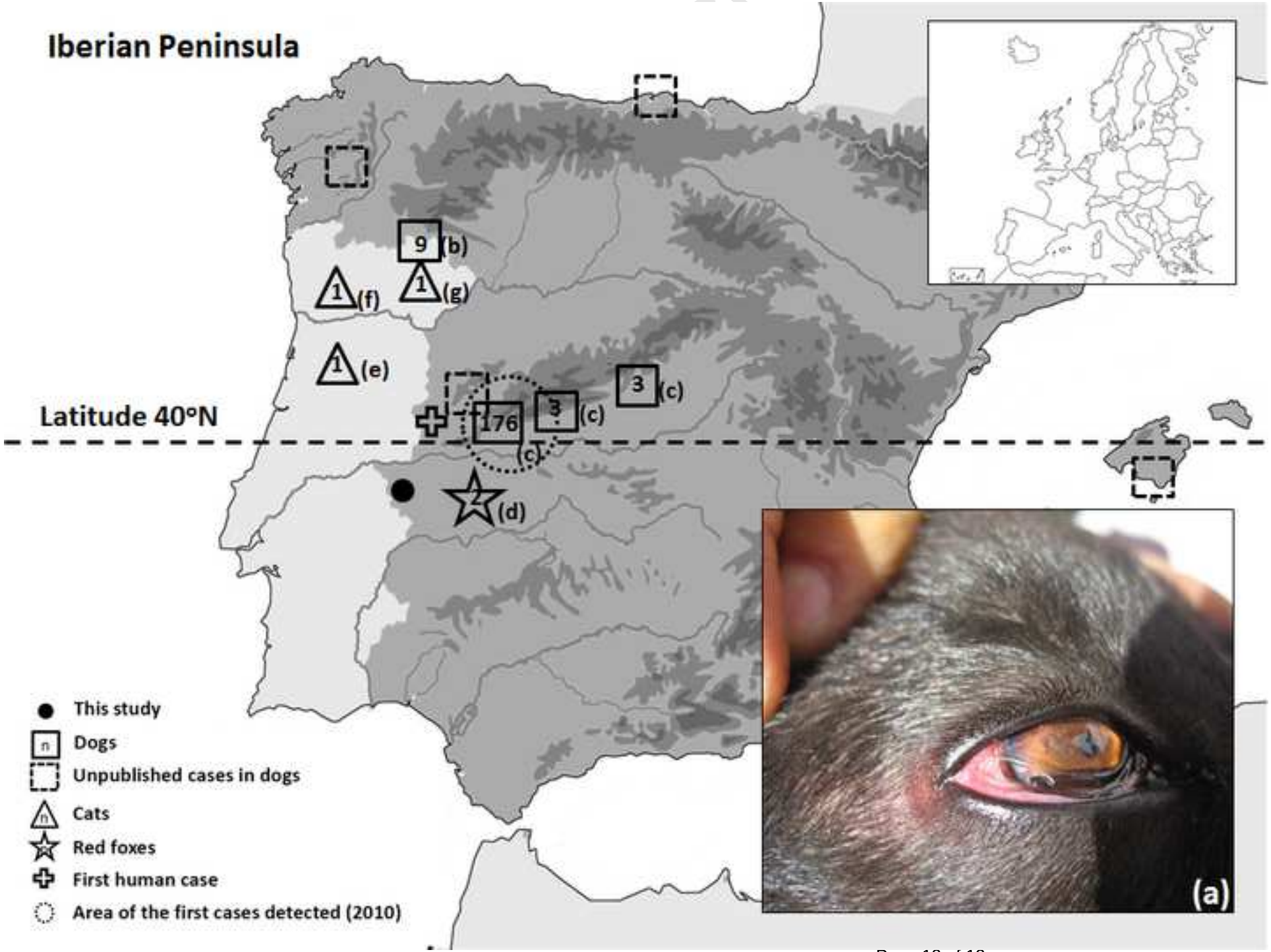
240 Fig. 1. Foci of thelaziosis by *Thelazia callipaeda* in the Iberian Peninsula. (a) *Thelazia*

241 worms on the eye of an affected Border Collie; Focuses: (b) reported by Vieira et al.

242 (2012); (c) by Miró et al. (2011); (d) by Calero-Bernal et al. (2013); (e) by Soares et al.

243 (2013); (f) by Pimenta et al. (2013); (g) by Rodrigues et al. (2012).

Figure 1


Iberian Peninsula
Latitude 40°N
9 (b)
1 (f)
1 (g)
1 (e)
3 (c)
176
3 (c)
(c)
(d)
(a)
This study
Dogs
Unpublished cases in dogs
Cats
Red foxes
First human case
Area of the first cases detected (2010)