

1 **Title:** International dog travelling and risk for zoonotic *Onchocerca lupi*

2 **Running Title:** Introduction of zoonotic *Onchocerca lupi*

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9 **Summary**

10 *Onchocerca lupi* is a recently recognized threat for the health of animals and humans in European,
11 American, African and Middle Eastern countries. We describe a case of imported *O. lupi* infection in Italy
12 and report the lifespan of this parasite in a non-endemic area, to advocate increased awareness of the
13 veterinary community for this zoonotic parasitosis.

14 **Keywords:** *Onchocerca lupi*; PETS; Travel medicine; Zoonoses; Animal travelling.

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16 **Main text**

17 Limited scientific knowledge of helminths affecting human and animal eyes challenge their diagnosis and
18 treatment (Otranto and Eberhard, 2011). Among these parasites, *Onchocerca lupi* is a zoonotic nematode
19 localising to the ocular and adnexal tissues of dogs, which act as reservoir hosts (Otranto et al., 2013). A
20 recent increase in *O. lupi* infection has been reported from dogs, cats, and humans living in European
21 (Romania, Hungary, Greece, Switzerland and Portugal), American (U.S. and Canada), Middle Eastern Asian
22 (Iran), and Northern African (Tunisia) countries (Gracío et al., 2015). Adult worms cause from mild ocular
23 lesions to blindness, but the majority of infected dogs do not display overt clinical signs (Otranto et al.,
24 2013). *Onchocerca lupi* has been in the limelight for its zoonotic potential (Otranto et al., 2011) and the
25 severe consequences upon the infections of children in the U.S. (Eberhard et al., 2013; Dudley et al., 2015).
26 The nonspecific tropism of this nematode for the CNS and the absence of resolute pharmacological
27 treatments entails hospitalization and invasive surgery for the removal of the parasite-embedding nodules in
28 humans (Eberhard et al., 2013; Dudley et al., 2015). The challenging diagnosis and the lack of information

29 on the species acting as vector, delay the establishment of surveillance and control strategies of *O. lupi*.
30 Here, we describe a case of imported *O. lupi* infection in Italy and provide information on the lifespan of this
31 parasite in a non-endemic area, to raise veterinary community's awareness.

32 In 2013 a female mixed breed dog was adopted in Italy from southern Portugal, an area classified as endemic
33 for canine onchocercosis (Otranto et al., 2013). The dog was referred to a practitioner in Italy for ocular
34 discomfort and subjected to an ultrasound examination (US) of both eyes and adnexal tissues (Franchini et
35 al., 2013). Hyper-echogenic structures were noticed in the eyes of the dog and removed from the sclera via
36 an exploratory surgery with the aid of US guidance (Franchini et al., 2013). The dog recovered without
37 complication and lived in Italy without further travelling abroad. The nodules were examined under a light
38 stereomicroscope, revealing the presence of adult *O. lupi* nematodes (Mutafchiev et al., 2013). Skin samples
39 were collected by using a disposable punch ($\approx 0.4 \times 0.5$ cm) from the interocular and interscapular regions of
40 the dog (Otranto et al., 2013), soaked in 0.9% saline solution for 12h at room temperature, and recovered
41 microfilariae morphologically identified as *O. lupi* (Otranto et al., 2013). Genomic DNA from the nodules
42 and from the microfilariae was extracted using a commercial kit (DNeasy Blood & TissueKit, Qiagen,
43 Germany) and molecularly processed for amplification and sequencing of the partial cytochrome oxidase
44 subunit 1 (*cox1*) gene, following procedures described elsewhere (Otranto et al., 2013). Nucleotide
45 sequences obtained from adults and skin DNA displayed 100% identity with those of *O. lupi* from Portugal
46 (GenBank accession no EF521410). In October 2017 the dog died from acute pancreatitis and was
47 necropsied. Non-encapsulated adult eyeworms were promptly recognized upon the enucleated ocular globe
48 (Figure 1), and morphologically identified as *O. lupi* adult nematodes. Skin samples were collected by
49 biopsy (≈ 0.5 cm) from the ears, nose, interocular and interscapular regions, which were analysed by
50 molecular means (as described above). Genomic DNA extracted from these parasites and skin biopsies of the
51 dog was amplified and were 100% identical to sequence from 2013 and with those of *O. lupi* from dogs in
52 Portugal (GenBank accession no EF521410 and MG677940). All study procedures on the dog were
53 performed after receiving the owner's informed consent.

54

55 International *O. lupi*-infected dog transportation poses a threat for the introduction of this zoonosis in non-
56 endemic areas and for the clinical consequences on human health. This nematode displays a very long

57 lifespan ranging from 3 (Verocai et al., 2016) to 8 years (Hodžić et al., 2017) in non endemic countries.
58 Similarly, *Onchocerca volvulus* can live in human nodules for approximately 15 years
59 (<https://www.cdc.gov/dpdx/onchocerciasis/index.html>). In this study *O. lupi* displayed a survival time of 4
60 years coupled with positivity of skin samples from several anatomical sites, which stands for the presence of
61 microfilariae in the dog's dermal tissues. This is paramount considering that *O. lupi* can live for a long time
62 and its offspring can be available to a vector species, even in non-endemic areas where the infection could
63 potentially establish. However, the risk for the introduction of *O. lupi* with travelling dogs cannot be
64 assessed in the absence of information about the presence of the vector(s) in a given area (Verocai et al.,
65 2016; Hodžić et al., 2017). Attempted treatments included various combination and dosages of melarsomine,
66 ivermectin, topical and systemic antibiotics, and oxfendazole, though studies proving the efficacy of these
67 compounds are lacking (Colella et al., *in press*). Likewise, surgical treatment did not prove successful in
68 curing canine onchocercosis, which is in line with previous reports of recurrent disease in dogs relocating
69 from endemic regions (Verocai et al., 2016; Hodžić et al., 2017). While recognizing the efforts of non-profit
70 organizations dealing with pet's adoption/relocation, an increased awareness of the veterinary community to
71 promptly diagnose canine onchocercosis, is fundamental to limit the introduction of *O. lupi* with
72 international transportation of animals from disease-endemic areas. Advocacy for a stronger commitment
73 should be also implemented by policy makers in Europe and U.S. to tackle the threat of this neglected
74 pathogen by making canine onchocercosis a reportable disease. For instance, the California Department of
75 Food and Agriculture has recently included onchocercosis by *O. lupi* in the disease priority list by reporting
76 the diagnosis within 7 days from time of identification
77 (<http://publichealth.lacounty.gov/vet/docs/2016LACountyAnimalReportableDiseaseList.pdf>).
78 Refined intervention strategies should consider the application of surveillance and proper treatment of *O.*
79 *lupi* infected dogs to avoid the introduction and/or spread of this life-threatening parasitic nematode.

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81 **Conflict of interest statement**

82 We declare that we have no conflict of interest.

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118 **Figure Legend**

119 Figure 1. *Onchocerca lupi* adult nematodes in the retrobulbar of the enucleated globe of the imported dog.