

CASE REPORT

A case of canine ocular onchocercosis in Portugal

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Abstract

Onchocercosis is a newly recognized disease in dogs that has been reported with higher frequency in Europe and in the United States. We report a case of a 3-year-old male mongrel stray dog from the Algarve region (South Portugal) who had a retrobulbar granuloma containing a filaroid nematode of the genus *Onchocerca*. A gravid adult female parasite was embedded in a granulomatous inflammation adjacent to the sclera beyond the retina. The parasite was 191 to 267 µm in diameter (mean = 225 µm), surrounded by a cuticle and owing a uterus that was filled with small unsheathed microfilariae. The cuticle consisted of two separated layers in longitudinal sections. The external layer had cuticular ridges and the internal layer contained striations. Sequencing of the COI and ND5 mitochondrial genes confirmed the identity of this parasite as *Onchocerca lupi*. Furthermore, the first sequence of the 12S mitochondrial gene is reported in this study.

Key Words: canine onchocercosis, filaridae, ocular histopathology, ocular parasitosis, onchocercosis, *Onchocerca lupi*

INTRODUCTION

Species of *Onchocerca* are common parasites of horses, cattle, and related ungulates,^{1–3} with the exception of the human parasite *Onchocerca volvulus*. Dogs and other canids, on the contrary, are not recognized to be natural hosts for any species of *Onchocerca*.

Sporadic cases of ocular and periocular *Onchocerca* infection have been reported recently in dogs in the United States, Hungary, Greece, and Germany but until now, there are only suppositions on the agent implicated.^{4–10} Some authors have addressed the hypothesis that the nematode found in the ocular or periocular regions in dogs are aberrant infections by *Onchocerca lienalis* in an accidental host with an ectopic location.^{4–6} *O. lienalis* is a filarial parasite that inhabits the connective tissue around the ligamentum nuchae, tibiofemoral ligament, spleen capsule, and other sites in cattle and buffalo. These authors support their hypothesis by the fact that the cuticular morphology of the female worm seen in those cases was very similar to the cuticular morphology of *O. lienalis*. They justify the different location of the parasite in both cattle and dog by the different feeding habitats of the vectors,¹¹ and argue that the small size of the microfilaria found in the uterus of the female worm and in

the surrounding tissues is due to the inappropriate conditions found in the accidental host.

Other authors claim, on the contrary, that this might be a new *Onchocerca* species infecting dogs as its natural host. They support their thesis by the fact that the natural location for *O. lienalis*, in cattle, was never observed in dogs and on the opposite course, ocular manifestation of cattle onchocercosis has never been reported either. They also state that the presence of large number of microfilaria found in the uterus of female worms and in the surrounding tissues contradicts the theory of being in an accidental host.^{7,8}

This article describes a new case of canine ocular onchocercosis observed in a dog from Portugal.

CASE

In August 2008, a 3-year-old male mongrel stray dog was presented to a local veterinarian, in Albufeira (Algarve, Portugal), with an ocular problem. On physical examination, the animal was apparently healthy, but showed signs of ocular pain and discomfort in the left eye. The OS presented blepharospasm and poor menace response. An open fistula with purulent discharge was noticed in the upper eyelid, causing alopecia of the skin along the draining tract (Fig. 1). The



Figure 1. An open fistula with purulent discharge was present in the dog's upper eyelid (arrow), causing alopecia of the skin along the draining tract.



Figure 2. The ophthalmologic exam revealed: a hyperemic and edematous conjunctiva, with purulent exudation on the medial canthus; corneal edema with mild neovascularization and a nonperforated descemetocele on its superior quadrant.

conjunctiva was severely hyperemic and edematous, with purulent discharge on the medial canthus. The cornea was severely edematous with mild neovascularization and a nonperforated descemetocele on its superior quadrant with fluorescein uptake. The severe corneal edema did not allow further examination of the ocular anterior and posterior compartments. (Fig. 2). Schirmer tear test and intraocular pressure, measured by applanation tonometry (TonoPen Vet, Medtronic Solan, Jacksonville, FL, USA) were within normal limits. The OD was unremarkable.

A scraping from the lesion was submitted for cytology and a corneal swab was taken for microbiology. The cytology slides presented normal corneal epithelial cells and nondegenerate neutrophils, and the microbiology culture grew *Enterobacter* spp. sensitive to cephalexin, gentamicin, and ciprofloxacin.

A complete blood count was performed and revealed leukocytosis ($20\,000/\text{mm}^3$) with neutrophilia ($15\,000/\text{m}^3$).

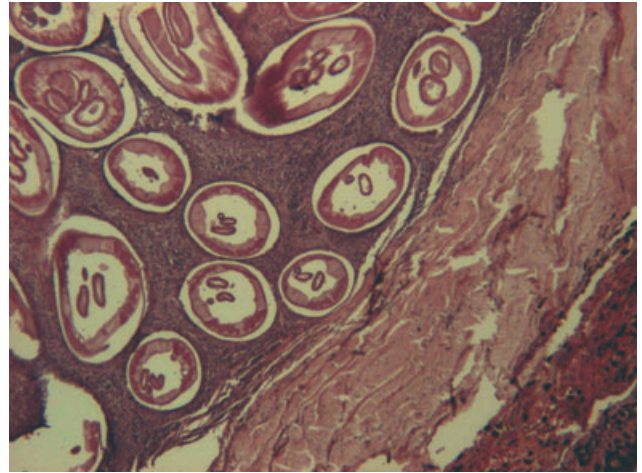


Figure 3. A granulomatous reaction with several sections of a coiled gravid female nematode in the retrobulbar space with 191–267 μm in diameter (H&E, $\times 40$).

Serologic diagnostic tests for *Leishmania infantum* (Leishmania spot IF, Ref 75931, Biomérieux, Lyon, France), *Dirofilaria immitis* (Eurovet veterinaria S.L. Ref D3213-AG01), *Babesia canis* (MegaScreen Fluobabesia Ref FB8225, MegaCor Diagnostik GmbH, Hörbranz, Austria), *Rickettsia conorii* (Vircell Rickettsia conorii slide, Vircell Microbiologists, Granada, Spain) and *Ehrlichia canis* (*E. canis* Agx12 Ref EC12, Fuller Laboratories, Fullerton, CA, USA) were conducted and only *B. canis* was mildly positive with a titer $\geq 1/32$.

The dog was treated with amoxicillin/clavulamic acid (22 mg/kg BID PO), cephalexin (30 mg/kg BID PO) and fucithalamic viscous eye drops (QID) for 1 week without showing any clinical improvement. An ecographic examination revealed a hyperechogenic mass with 0.5 cm in the long axis in the retrobulbar space. The dog went to surgery for an enucleation procedure because of the poor eye condition.

The eye was removed and fixed in 10% phosphate buffered formalin, and hematoxylin and eosin (H&E) stained sections were prepared.

Histopathological examination of the mass revealed a granulomatous reaction with several sections of a coiled gravid female nematode in the retrobulbar space in close contact with the outer layer of the sclera (Fig. 3). The inflammatory exudate was rich in macrophages and neutrophils followed by plasmacytes. Several microabscesses surrounded the body of the nematode.

The parasite was 191–267 μm in diameter (mean = 225 μm), surrounded by a cuticle within which an intestine was observed. In some transverse sections of the parasite, the uterus was detected and was filled with small unsheathed microfilariae (Fig. 4). The cuticle consisted of two separated layers in longitudinal sections. The external layer had cuticular ridges (annulations) that appeared as cuticular bumps in longitudinal sections. The ridges were round in shape, 3–5 μm tall, evenly spaced and distanced 22 μm from each

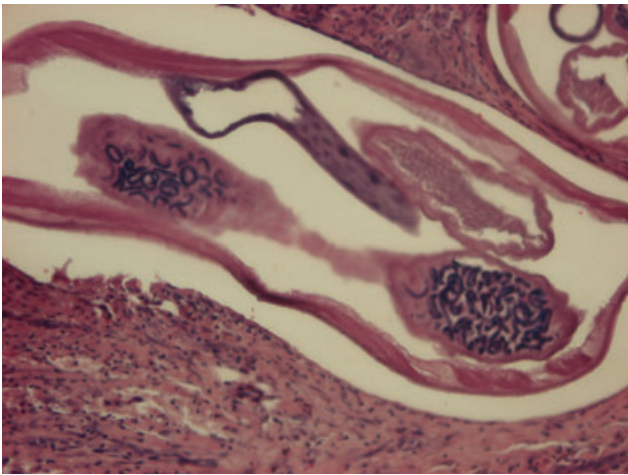


Figure 4. Transverse section of the female worm owing a uterus filled with small microfilariae (H&E, $\times 100$).

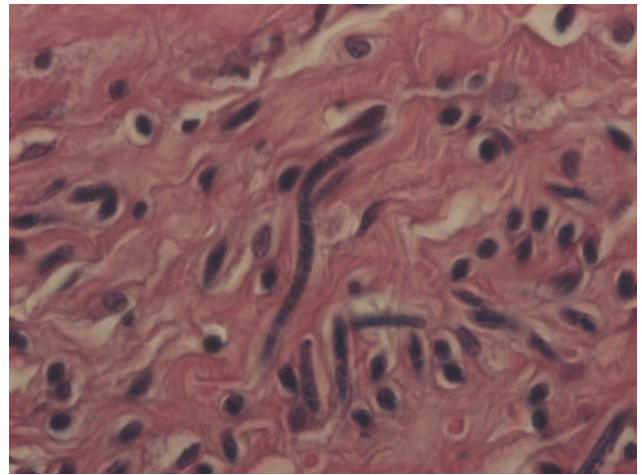


Figure 6. In the surrounding sclera, there were numerous exceptionally small free microfilariae (H&E, $\times 400$).

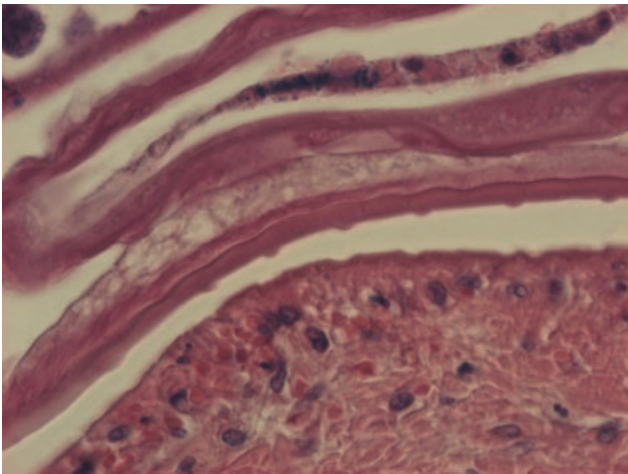


Figure 5. The cuticle consisted of two separated layers in longitudinal sections. The external layer had cuticular ridges that were round in shape, 3- to 5- μm tall, evenly spaced and distanced 22 μm from each others (arrows), and the internal layer contained on average one striation under every ridge and one between neighboring ridges (H&E, $\times 400$).

others (Fig. 5). The internal layer below the ridges contained striations, on average one striation under every ridge and one between neighboring ridges. The cuticle was about 6- μm thick between ridges and 11 μm at ridges. The lateral chords were marked, and the nuclei of the chord were ovoid, measuring $9 \times 7 \mu\text{m}$. In the surrounding sclera, numerous free microfilariae were observed, that measured on average 70- μm long by 5- μm thick (Fig. 6).

In spite of the severe inflammation and numerous microfilariae in the fibrous outer tunic of the globe, the choroid, retina, and vitreous space adjacent to the granuloma were unaffected. The corneal ulcer was probably self-inflicted due to the discomfort and ocular pain and secondarily infected with *Enterobacter* spp.

MOLECULAR DIAGNOSTIC

DNA was extracted from a fragment of one adult parasite using the QIAamp DNA Mini Kit from QIAGEN (Izasa Portugal, Lda, Carnaxide, Portugal). Three mitochondrial genes (COI, ND5 and 12S rDNA) were amplified using primers described in Morales-Hojas *et al.* (2006) and Casiraghi *et al.* (2001) (the nondegenerate primers COIintF and COIintR).^{12,13} PCRs were performed in a total volume of 15 L containing 1 \times buffer, 3 mM MgCl_2 , 200 M of each dNTP, 0.5 M of each primer, 1 unit of *Taq* DNA polymerase and 3 L of the extracted DNA. Amplifications consisted of a first denaturation step at 94 $^\circ\text{C}$ for 2 min 30 s followed by 35 cycles of 30 s at 94 $^\circ\text{C}$, 45 s at 50 $^\circ\text{C}$ and 45 s at 72 $^\circ\text{C}$, with a final extension step of 5 min at 72 $^\circ\text{C}$. Amplification products were extracted from an agarose gel using the QIAEX II Agarose Gel Extraction kit from QIAGEN. PCR products were cloned using the TOPO TA Cloning Kit for Sequencing from Invitrogen (Barcelona, Spain) and plasmids were extracted from positive colonies using the QIAprep Spin Miniprep Kit from QIAGEN. Three clones were sequenced for each gene in order to correct for PCR and cloning misincorporations. Cycle sequencing was performed using ABI Big Dye v1.1 chemistry with the universal M13 primers as sequencing primers. Sequencing products were run at StabVida Inc. (Lisbon, Portugal). DNA sequences were checked for errors with BioEdit v5.09 (Hall, T., Ibis Biosciences, Carlsbad, CA, USA) and have been deposited in GenBank with accession numbers GU365877, GU365878, GU365879, for COI, ND5 and 12S genes, respectively. The COI and ND5 sequences were compared with the GenBank dataset using the BLAST programme (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>), which identified the other sequences of *O. lupi* as having the highest identity to the sequence obtained in this study. Genetic distances between sequences were estimated with MEGA 4. The genetic p-distances of the *O. lupi* sequence obtained in this

study to the other *O. lupi* sequences ranged from 0.2% to 1.7% at the ND5 gene and from 0% to 2% at the COI. The *O. lupi* from Portugal was the closest sequence to that obtained in this study. The evolutionary divergence estimated using the Maximum Composite Likelihood between *O. lupi* and other *Onchocerca* spp. (including *O. lienalis*) ranged from 6.8% to 10% at the ND5 and from 8.8% to 12% at the COI. The evolutionary divergence between *O. lupi* and *D. immitis* was 20% for the ND5 and 13.2% for the COI. The 12S gene sequence obtained in the present analysis is the first *O. lupi* sequence deposited in GenBank for this species. The evolutionary divergence estimated using the Maximum Composite Likelihood between the *O. lupi* 12S sequence and the other *Onchocerca* spp. (also including *O. lienalis*) ranged from 4.8% to 8.1%, whereas to *D. immitis* was 13.7%. Furthermore, phylogenetic reconstructions with the ND5 and COI gene sequences using Maximum Parsimony placed our sequence in the same clade as the other *O. lupi* sequences with high bootstrap values (results not shown). Given these results, it is clear that the parasite obtained from the mongrel stray dog belonged to the species *O. lupi*.

DISCUSSION

Several canine ocular helminthic infections have been described so far. Some of them represent ectopic location of natural parasites in dogs, such as immature adults of *D. immitis*¹⁴ and larvae of *Angiostrongylus vasorum*¹⁵ that were observed in the anterior chamber of two different dogs and a case of retinitis and intraocular larval migration in a group of Border collies caused by *Toxocara canis*.¹⁶

The case here described has similar features in terms of location (retrobulbar space, and conjunctiva), type of inflammatory reaction, and parasite morphology to the previously described cases of canine onchocercosis, suggesting that it is caused by the same parasitic species. The unique parasite morphology showing a two-layer cuticle with ridges on the external side and striations on the internal layer is exclusive of *Onchocerca* spp. The distinction between *Onchocerca* species is also based on the cuticular morphology of female worms.^{1,17–21} The cuticle of this female worm with one striation per ridge and one striation between ridges is very peculiar and was identical to the ones observed in the other cases of canine onchocerciasis in the United States, Greece and Hungary.^{4–7,9} The only two *Onchocerca* species known that have a similar cuticle are *O. lienalis* of cattle and *O. volvulus* of man.¹ The small size of the microfilariae found here is also a peculiar characteristic of canine onchocercosis cases, as all the other *Onchocerca* spp. have consistently longer microfilariae.²² In this case, the morphological characteristics associated with DNA sequencing and phylogenetic analyses identified the parasite as *O. lupi*.

Onchocerca lupi has recently been re-described based on morphological and genetic characteristics,^{23,24} in comparison with the first original description in 1967 by Rodonaja *et al.*²⁵ *O. lupi*, however, is not listed in the official list and

index of the International Commission on Zoological Nomenclature. To be fully accepted as a species, a formal taxonomic description with deposition of a type specimen in a Museum of Natural History is required.

Although it is not the intention of this study to enter a taxonomic discussion about the validity of the name or its specific status, it is important to compare the parasite detected in this study with previous recent reports in the literature. BLAST comparisons of our sequences resulted in highest homology to sequences deposited before, in GenBank, and identified as *O. lupi*. Phylogenetic analyses also resulted in the clustering of our sequences with those reported as *O. lupi* by other authors.²⁴ Therefore, we can conclude that our parasite is the same species as those previously reported as *O. lupi* (independently of the name validity).

The discussion remains, but it is clear that the number of reported cases of canine onchocercosis is steadily increasing and the geographic distribution is becoming wider as seen in this article with a new case in Portugal. The authors also state that as happened in this case, it is important to start considering infection with *Onchocerca* in the work up of ocular and periocular nodules, in Portugal, to avoid mistaking them for abscesses or tumors.

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