

Autochthonous, zoonotic *Onchocerca lupi* in a South Texas dog, United States

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Abstract

Onchocerca lupi is an emerging, zoonotic filarioid nematode associated with ocular disease in companion animals. We report the first autochthonous case of *O. lupi* in a dog from Hidalgo County, southern Texas, USA. An 11-year-old, castrated male, Pitbull dog from McAllen, Hidalgo County, southern Texas with no travel history, was diagnosed with a perforating corneal ulceration of the right eye. Enucleation was performed, and tissues submitted for histopathology. Sections of two filarioid nematodes were observed. Parasite identification relied on morphological features seen on histopathology and molecular confirmation by PCR of the *cox1* gene of the mitochondrial DNA, followed by sequencing and phylogenetic analysis. Our finding suggests Texas as an additional endemic state for this zoonotic nematode. Further investigations are required to understand the epidemiology of this parasite along the United States/Mexico border.

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Summary

Onchocerca lupi is an emerging, zoonotic filarioid nematode associated with ocular disease in companion animals. We report the first autochthonous case of *O. lupi* in a dog from Hidalgo County, southern Texas,

USA. An 11-year-old, castrated male, Pitbull dog from McAllen, Hidalgo County, southern Texas with no travel history, was diagnosed with a perforating corneal ulceration of the right eye. Enucleation was performed, and tissues submitted for histopathology. Sections of two filarioid nematodes were observed. Parasite identification relied on morphological features seen on histopathology and molecular confirmation by PCR of the *cox1* gene of the mitochondrial DNA, followed by sequencing and phylogenetic analysis. Our finding suggests Texas as an additional endemic state for this zoonotic nematode. Further investigations are required to understand the epidemiology of this parasite along the United States/Mexico border.

Key words: Filarioidea, Ocular onchocercosis, Vector-borne diseases, Zoonotic onchocerciasis

1. Introduction

Onchocerca lupi (Nematoda: Onchocercidae) is an emerging, zoonotic parasite in North America and areas of the Old World (Cantey et al., 2016; Eberhard et al., 2013; Grácio, Richter, Komnenou, & Grácio, 2015; Otranto et al., 2015). Infection in dogs and cats are usually associated with episcleral and periocular tissues causing minor to severe ocular lesions, including conjunctivitis, third eyelid prolapse, exophthalmos, uveitis, and retinal detachment (McLean, Newkirk, & Adema, 2017; Otranto et al., 2015). However, the presentation of zoonotic onchocerciasis by *O. lupi* in humans may be variable according to the site of infection.

To date, there have been seven confirmed zoonotic onchocerciasis cases by *O. lupi* in the United States. Six of these cases involved children (Cantey et al., 2016; Eberhard et al., 2013). An additional medical concern is that three of these cases were associated with nodules at the cervical spinal cord; two of these cases had gravid adult female specimens, suggestive that these were patent infections. These North American human *O. lupi* infections have been reported from Arizona, New Mexico, and Texas. While, Arizona and New Mexico are considered endemic for this parasite, with multiple cases reported from companion animals, humans and wildlife (Cantey et al., 2016; McLean et al., 2017; Roe et al., 2020); the origin of the single human case in southern Texas remains uncertain. Thus far, all canine cases in North America reported outside of the presumed endemic southwestern United States (e.g., Minnesota, Florida, New York) have been associated with travel (Edelmann, Jager, Espinheira, & Ledbetter, 2018; Otranto et al., 2015; Verocai et al., 2016). Similarly, some European cases in dogs and humans have been linked to translocation or travel to endemic regions of the Mediterranean (Colella et al., 2018; Hodžić et al., 2018; Sandell, Skogen, Lier, & Pettersen, 2020).

2. Materials and Methods

In August 2020, an 11-year-old, castrated male, Pitbull dog from McAllen, Hidalgo County, southern Texas, was presented to the veterinarian with a history of ocular irritation for approximately 2-3 weeks. It was diagnosed with a perforating corneal ulceration of the right eye. The dog was born in McAllen and had never left the Rio Grande River Valley, Texas. The animal tested antigen-positive for heartworm, *Dirofilaria immitis*, during pre-anesthetic work-up, despite somewhat compliant chemoprophylaxis with an ivermectin-based monthly product. An enucleation was performed, and the eye was fixed in 10% neutral buffered formalin and submitted to the Texas A&M Veterinary Medical Diagnostic Laboratory for histologic processing and examination.

Genomic DNA was extracted using a Qiagen FFPE Tissue DNA extraction kit (Qiagen, USA) according to manufacturer's instructions. Polymerase chain reaction (PCR) was performed in 25 µL reactions containing 0.25µM of each primer, 1x GoTaq® Green Master Mix (Promega Corporation, Madison, Wisconsin, United States) and 2.5 µL of DNA template. The cytochrome oxidase c subunit 1 (*cox1*) gene was amplified using the forward primer COIntF: 5'-TGA TTG GTG GTT TTG GTA A-3' and reverse primer COIntR: 5'-ATA AGT ACG AGT ATC AAT ATC- 3' (Casiraghi, Anderson, Bandi, Bazzocchi, & Genchi, 2001; Otranto et al., 2015; Otranto et al., 2011). The cycling conditions included an initial denaturation step at 95 for 2 minutes, followed by 40 cycles at 95°C for 45 seconds, 52 at 45 seconds, and 72 for 90 seconds, and a final extension step at 72degC for 5 minutes. PCR products were purified using E.Z.N.A.^(r) Cycle Pure Kit (Omega Bio-tek, Norcross, GA, USA) according to the manufacturer's instructions, followed by Sanger sequencing. Phylogenetic analysis was performed in MEGA X using the Maximum Likelihood method and

General Time Reversible, Gamma distributed as the best fit model (Kumar, Stecher, Li, Knyaz, & Tamura, 2018).

3. Results and Discussion

Our report represents the first unequivocally autochthonous case of *O. lupi*, an agent of zoonotic onchocerciasis, in Texas, near the United States/Mexico border based on integrated histopathological, parasitological and molecular data.

Histologically, the most significant finding was the presence of corneal perforation with severe keratitis and anterior iris synechiae. Additionally, two long, filarioid nematodes were discovered embedded in the episcleral tissues. One of these parasites was degenerated and surrounded by granulomatous inflammation, while the other was intact and lacked surrounding inflammation. This specimen was a gravid, female nematode, suggesting a patent infection. The pattern of cuticular ridges of two inner striae within the space between two outer cuticular ridges observed on the specimens were morphologically consistent with *O. lupi* (Verocai et al., 2016) (Figure 1). Most likely, the dog was co-infected with heartworm, *D. immitis*, as a recent study, has shown that *O. lupi* infections are unlikely to generate false-positive results in commercial heartworm antigen tests (Sobotyk Oliveira, Savadelis, McLean, & Verocai, 2020).

The generated *cox1* sequence was accessioned in GenBank (MW577256), and showed 99.9 – 100% maximum identity with *O. lupi* sequences available in GenBank. Phylogenetic analysis clustered the Texas isolate with all previous isolates from North America, and some European isolates, belonging to *O. lupi* “genotype 1” (99% bootstrap support; Figure 2) (Rojas, Salant, Yasur-Landau, Tsarfati, & Baneth, 2020).

Zoonotic onchocerciasis has been previously reported from a 10-year-old boy from Mission, Texas also in the Hidalgo County, who traveled to South Dakota, and slept in tents and cabins in New Mexico and Colorado, reported fishing in fresh water lakes near home, and had a pet dog with history of conjunctivitis and eye lesion of unknown etiology (Cantey et al., 2016). Altogether, the present canine case may suggest that this human infection may have been, in fact, acquired in Texas.

It is necessary to better understand the epidemiology of *O. lupi* in this newly recognized endemic area by screening dogs and cats from shelters using classical and molecular methods. In addition to companion animals and humans, coyotes (*Canis latrans*) were reported infected and may serve as wild reservoirs in the western United States (Roe et al., 2020), and may also contribute to the epidemiology of *O. lupi* in southern Texas, and neighboring Mexico. Other wild carnivores that are known or have postulated as *O. lupi* hosts such as wolves (*Canis lupus*) and certain foxes (red fox, *Vulpes vulpes*; swift fox, *Vulpes velox*; kit fox, *Vulpes macrotis*) are not present in southern Texas. However, the widely distributed gray fox (*Urocyon cinereoargenteus*), is reported in this region, and across *O. lupi* endemic areas in North America, and therefore, should be assessed as potential reservoir host. Regarding the dipteran vectors, black flies (Simuliidae) have been considered the putative intermediate host for *O. lupi* (Hassan et al., 2015); however, biological confirmation remains necessary. Therefore, xenomonitoring in Texas could also involve screening of other dipterans, such as biting midges (Ceratopogonidae: *Culicoides*) and sand flies (Psychodidae: Phlebotominae).

This unequivocal autochthonous *O. lupi* case in southern Texas suggests that this parasite is endemic to the region, and transmission may occur locally. In this case, it is postulated that *O. lupi* could have caused exophthalmos with secondary traumatic corneal ulceration and subsequent perforation. There is a need for epidemiological surveillance of companion animals, wildlife, and dipteran vectors along the United States/Mexico border. It is important to raise awareness of public health and medical specialists and authorities as humans in this region might be at risk of infection.

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Dr. Sobotyk is the NCVP Merck Parasitology Resident at Texas A&M University.

Conflict of interest statement

The authors declare no conflict of interest.

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Figure legends

Figure 1: Histologic section with a gravid, female *Onchocerca lupi* specimen embedded in the episclera of a dog from South Texas. The cuticular pattern of two inner striae (dashed arrows) within the space between two outer cuticular ridges (solid arrows) aided in the species—level identification. (H&E, 40X, Bar = 50µm).

Figure 2: Phylogenetic relationship of based on *cox1* gene of *Onchocerca lupi* nematode isolate from a dog in South Texas, United States (GenBank accession no. MW577256), and other *Onchocerca* spp. isolates. Analysis was performed using the Maximum Likelihood method (1,000 bootstrap replicates). Nodes with less than 90% support were condensed.



