Onchocerca lupi Nematode in Cat, Portugal

Carla Maia, Giada Annoscia, Maria Stefania Latrofa, André Pereira, Alessio Giannelli, Laurentina Pedroso, Domenico Otranto

Author affiliations: Universidade Nova de Lisboa, Lisbon, Portugal (C. Maia); Universidade Lusófona de Humanidades e Tecnologias, Lisbon (C. Maia, A. Pereira, L. Pedroso); Università degli Studi di Bari, Valenzano, Italy (G. Annoscia, M.S. Latrofa, A. Giannelli, D. Otranto)

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To the Editor: *Onchocerca lupi* (Spirurida, Onchocercidae) is a nematode that infects the ocular tissues of dogs and humans. This filarioid remained almost unknown until recently, when it was reported in dogs from Europe and North America (*1*–*3*). *O. lupi* was also detected in 2 cats from the United States (*4*), which suggests that not only canids but also felids are suitable hosts for this little-known nematode. In addition, the zoonotic potential of *O. lupi* nematodes was demonstrated in human patients from Iran, Tunisia, Turkey, and the United States (*3*,*5*).

Clinical signs of canine onchocercosis include conjunctivitis, exophthalmos, periorbital swelling, photophobia, discomfort, lacrimation, ocular discharge, subconjunctival granuloma, ulcerative keratitis, and anterior and posterior uveitis (*1*). Signs in cats are similar to those in dogs (*4*).

After the first case of canine ocular onchocercosis was reported in the Algarve region in southern Portugal (*6*), a survey to detect microfilariae in apparently healthy dogs revealed an 8.3% prevalence of infection (*7*). Because no data regarding *O. lupi* nematode infection in cats from Europe are available, the aim of this study was to evaluate the infection's occurrence in cats in Portugal, where canine infection has been previously reported (*8*).

In October 2014, a total of 155 stray cats were sampled from Praia de Faro in the Algarve (37°0′29.4546″N, 7°59′41.265″W, altitude 9 meters). The sampling area is a small peninsula within an area characterized by a line of sand dunes formed by peninsulas and sandy islands that protect a vast area of marshland, canals, and islets from the Atlantic Ocean. All stray cats were captured under the scope of a trap, neuter, and return project. This study was

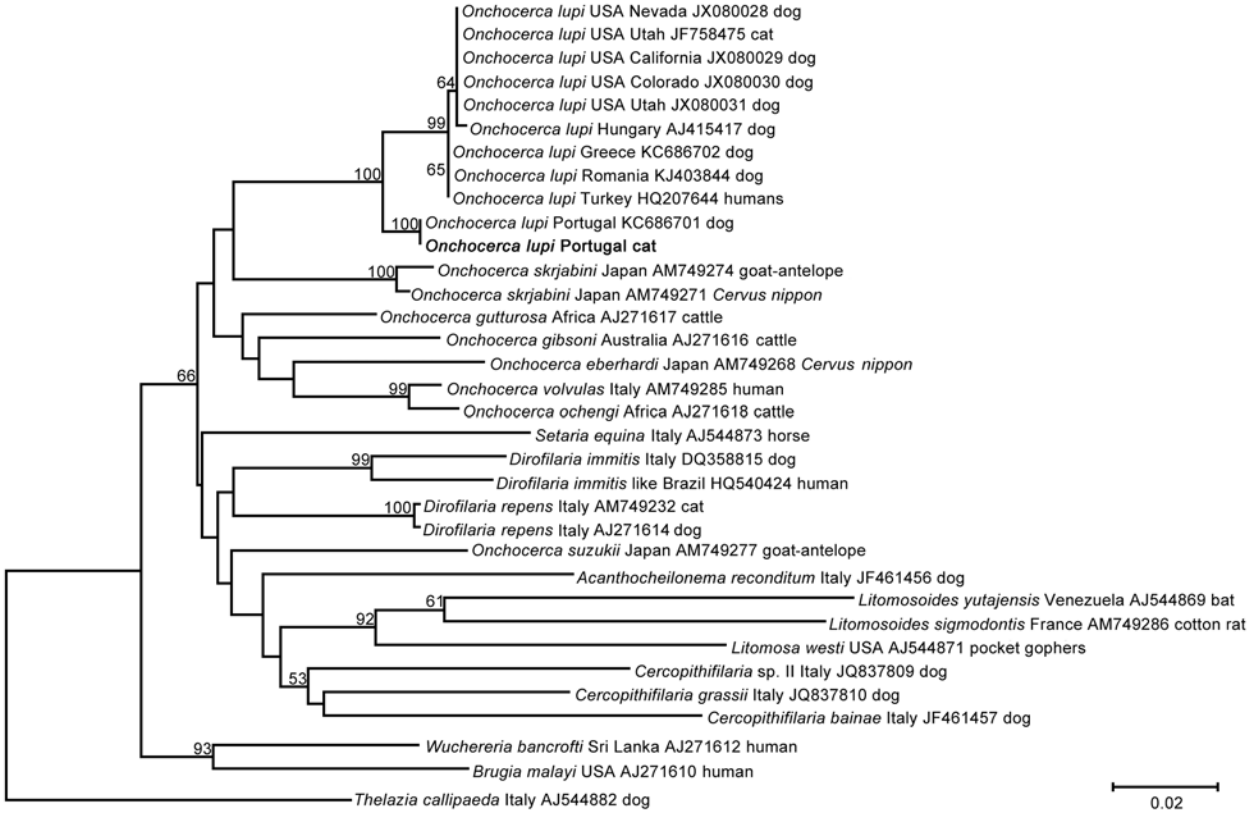

Figure. Phylogenetic analysis of partial cytochrome *c* oxidase subunit 1 gene segment (689 bp) of *Onchocerca lupi* isolated from a cat in Portugal (bold) compared with segments from other nematodes and roundworms retrieved from GenBank (accession numbers indicated). Numbers along branches are bootstrap values. Scale bar indicates nucleotide substitutions per site.

approved by the ethical committee of the Faculty of Veterinary Medicine, Universidade Lusófona de Humanidades e Tecnologias.

Ear tipping is commonly done in trap, neuter, and return programs to identify cats that have been sterilized. These skin samples (0.5 cm^2) were soaked at room temperature in 1 mL of saline solution, and sediments were individually observed under light microscopy (*9*).

Of 155 cats, 1 (0.65%) with no clinical signs of ocular infection was positive for *O. lupi* microfilariae. Microfilariae were identified according to morphologic keys (*9*) and differentiated from those of other filarioid species infecting cats in the Mediterranean region. *O. lupi* microfilariae had a short, flattened, unsheathed body (mean length 110.1 ± 7.5 μm, width 6.8 ± 1.2 μm) with a rounded head bearing a tiny tooth on the cephalic edge. The body was blunt with a short bent tail of ≈11.7 μm.

After we made microscopic observations, skin samples were processed as described elsewhere (*10*). Partial cytochrome *c* oxidase subunit 1 (*cox*1) gene fragments (689 bp) were amplified (*10*). In accordance with the morphologic identification, BLAST analysis (http://blast.ncbi.nlm.nih.gov/Blast.cgi) of *cox*1 gene showed a high overall nucleotide homology with sequences of *O. lupi* available in GenBank. All *cox*1 sequences available in GenBank for *O. lupi* nematodes were analyzed by using MEGA6 (http://www.megasoftware.net) and showed a low intraspecific variability, ranging from 0% to 2.1% (mean 0.7%). Phylogenetic analysis of *cox*1 sequences with MEGA6 and the neighbor-joining method confirmed that the sequence obtained clustered with that of *O. lupi* nematodes from Portugal available in GenBank (Figure). The obtained sequence was deposited in GenBank (accession no. KP453715).

We describe detection of *O. lupi* nematodes in a cat from Europe. The complete life cycle of *O. lupi* nematodes remains unknown, although arthropods should act as a vector (*2,4,7*). Because most of the potential vectors (i.e., black flies, mosquitoes, and biting midges) increase their activity during spring and summer, we cannot rule out that skin sampling conducted in late October affected the chance to detect additional infected animals. In addition, sampling was performed during the day, instead of late afternoon or night, when the number of microfilariae is higher (*7*), which might account for the low prevalence of infection obtained in this study.

As previously reported for most infected dogs from the same area, the infected cat lacked apparent clinical signs of infection, suggesting that subclinically infected animals might be carriers and reservoirs of *O. lupi* nematodes (*7*). Further investigation such as population-based surveys should be performed to estimate the distribution of the infection in cats and dogs and to assess the risk to humans.

Detection of *O. lupi* nematodes in dogs and cats from Algarve confirms that this parasite is endemic to southern Portugal. Veterinarians, local pet owners, and tourists (particularly those from countries where the disease is not endemic and who bring their pets) should be alerted to the risk for infection by this filarioid and the need to implement measures to protect animals and persons. Physicians and ophthalmologists should include this zoonosis in the differential diagnosis for ocular nodular lesions, particularly in patients from areas where *O. lupi* nematodes have been reported.

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Address for correspondence: Carla Maia, Global Health and Tropical Medicine, Medical Parasitology Unit, Institute of Hygiene and Tropical Medicine, New University of Lisbon, Lisbon, Portugal; email: carlamaia@ihmt.unl.pt

Porcine Deltacoronavirus in Mainland China

Nan Dong, Liurong Fang, Songlin Zeng, Qianqian Sun, Huanchun Chen, Shaobo Xiao

Author affiliation: Huazhong Agricultural University, Wuhan, China (N. Dong, L. Fang, S. Zeng, Q. Sun, H. Chen, S. Xiao); The Cooperative Innovation Center for Sustainable Pig Production, Wuhan (L. Fang, H. Chen, S. Xiao)

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To the Editor: Porcine deltacoronavirus (PDCoV) was discovered in 2012, during a study to identify new coronaviruses in mammals and birds in Hong Kong (*1*). In February 2014, this novel porcine coronavirus was detected in pigs in Ohio, United States (*2*), and has since been reported in at least 17 US states (*3–5*). Concern regarding the epidemiology, evolution, and pathogenicity of this emerging virus is increasing. Recently, PDCoV was identified in South Korea (*6*). We report PDCoV in mainland China.

Since December 2010, a large-scale outbreak of diarrhea in suckling piglets has occurred on swine farms in mainland China (*7*). The causative agent was considered to be a variant of porcine epidemic diarrhea virus (PEDV) (*8*), and the role of PDCoV in the outbreak was not investigated at that time.

Using 2 pairs of specific primers to detect PDCoV, as described by Wang et al. (*2*), we tested 215 intestinal or fecal samples collected at various times during 2004–2014 from piglets with clinical diarrhea in Anhui, Guangxi, Hubei, and Jiangsu provinces, mainland China (online Technical Appendix Table 1, http://wwwnc.cdc.gov/EID/article/21/12/15-0283-Techapp1.pdf). All samples were submitted from commercial pig farms to our laboratory for enteropathogen detection. Of these samples, 165 (124 from Hubei, 41 from Jiangsu) had been collected in 2014, and 50 (40 from Jiangsu, 6 from Anhui, 4 from Guangxi) had been collected during 2004–2013 and preserved in our laboratory. The 215 samples were simultaneously tested for PEDV and transmissible gastroenteritis virus (TGEV) by using the primers listed in online Technical Appendix Table 2. Of the samples tested, 14 (6.51%) were positive for PDCoV, 110 (51.2%) were positive for PEDV, and 5 (2.3%) were positive for TGEV. Of the 14 PDCoV-positive samples, 7 (50%) were also positive for PEDV; 2 of the 215 samples were co-infected with PEDV, TGEV, and PDCoV (online Technical Appendix Table 1). Previous studies have shown that prevalence of PDCoV in the midwestern United States in 2014 was high (30%) and that PDCoV co-infections with other pathogens (such as PEDV and rotavirus) are more common (78% of PDCoV infections) (*4*). At the same time in mainland China, the rate of PDCoV positivity was lower (7.27%), whereas that of PEDV was higher (52.73%), suggesting that PEDV remains the main causative agent of piglet diarrhea diseases in mainland China. Similarly, in South Korea in 2014, only 2 PDCoV-positive samples were detected in 113 samples of diarrhea from pigs (*6*).

We also examined the collection dates and geographic locations of the PDCoV-positive samples and found that PDCoV was detected in pigs in Hubei (8/124), Jiangsu (4/81), and Anhui (2/6) Provinces. However, all samples from pigs in Guangxi Province were negative for PDCoV. All PDCoV-positive samples from Hubei and Jiangsu Provinces had been collected in 2014, whereas the 2 PDCoV-positive samples from Anhui Province had been collected in 2004.

Among the PDCoV-positive samples, we selected 3 for complete genome sequencing with 16 pairs of overlapping primers, as described previously (*2*): one (CHN-AH-2004) collected from Anhui Province in 2004, one (CHN-HB-2014) from Hubei Province in 2014, and one (CHN-JS-2014) from Jiangsu Province in 2014. These complete genome sequences have been deposited in GenBank under accession nos. KP757890 (CHN-AH-2004), KP757891 (CHN-HB-2014), and KP757892 (CHN-JS-2014). The complete genome sequences of 3 PDCoV strains from pigs in mainland China shared high nucleotide identities (≥98.9%) with all previously reported PDCoV strains. Previous studies found that Hong Kong strain HKU 15–44 and all PDCoV strains from the United States and South Korea have a 3-nt insertion in the spike gene, which is not present in Hong Kong strain HKU 15-155 (*2–6*). This insertion is also present in CHN-AH-2004, whereas CHN-HB-2014 and CHN-JS-2014, like HKU 15-155, lack this insertion (online Technical Appendix Figure).

Although all reported PDCoV strains from China shared high similarity with each other, a phylogenetic tree based on all available complete PDCoV genome sequences showed that these PDCoV strains clearly cluster in different clades (Figure). Strain CHN-JS-2014 shares an ancestor with the strains from the United States and South Korea. CHN-AH-2004 and HKU15-44 share a common ancestor, and CHN-HB-2014 shares a common ancestor with CHN-AH-2004 and HKU15-44.

As an emerging virus, PDCoV has been poorly understood. Our data suggest that PDCoV has existed in