International Journal for Parasitology: Parasites and Wildlife 24 (2024) 100954

Contents lists available at ScienceDirect

International Journal for Parasitology: Parasites and Wildlife

journal homepage: www.elsevier.com/locate/ijppaw

Dirofilaria immitis and *Dirofilaria striata* (Spirurida: Onchocercidae) detected in wild carnivores from Texas, United States

Rafael A.N. Ramos [a,b], Hassan Hakimi [a], Jordan Salomon [c], Rachel E. Busselman [c], Rachel Curtis-Robles [c], Carolyn L. Hodo [a,d], Sarah A. Hamer [c], Guilherme G. Verocai [a,*]

[a] Department of Veterinary Pathobiology, School of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX, 77843, USA
[b] Laboratory of Parasitology, Federal University of the Agreste of Pernambuco, Garanhuns, PE, 55282-278, Brazil
[c] Department of Veterinary Integrative Biosciences, School of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX, 77843, USA
[d] Department of Comparative Medicine, The University of Texas MD Anderson Cancer Center, Bastrop, TX, 78601, USA

ARTICLE INFO

Keywords:
Coyote
Raccoon
Bobcat
Vector-borne
Wild reservoir
Zoonosis

ABSTRACT

Dirofilaria immitis and *Dirofilaria striata* (Spirurida: Onchocercidae) are epidemiologically important filarial nematodes detected in wild carnivores sympatric to domestic animals and humans. In this study we surveyed for *Dirofilaria* species among previous studies archived blood samples (n = 202) of wild carnivores sourced across Texas between the years of 2014–2016 and 2020 to 2023. In total, 117 coyotes (*Canis latrans*), 67 raccoons (*Procyon lotor*), 12 gray foxes (*Urocyon cinereoargenteus*), five bobcats (*Lynx rufus*), and one striped skunk (*Mephitis mephitis*) were tested through the amplification of the partial cytochrome oxidase c subunit 1 (*COI*) gene followed by sequencing. *Dirofilaria immitis* was detected in 11.39% (95% CI = 7.71–16.51) of the samples (21 coyotes and two raccoons), while *D. striata* was detected in a single bobcat. *Dirofilaria immitis* sequences had 99.85%–100% (99.92% ± 0.08) similarity with other *D. immitis* sequences in GenBank. The sequence of *D. striata* from the bobcat was 100% similar to the single *COI* sequence available in GenBank. Data from this study reinforce the role of coyotes as a wild reservoir for *D. immitis* and suggest that raccoons may also play a role in the epidemiology of this parasite. This study additionally provides molecular data on *D. striata*, an understudied filarioid of felids.

1. Introduction

Filarial parasites are important vector-borne helminths in domestic and wild animals worldwide (Otranto and Deplazes, 2019; Gruntmeir et al., 2023), with those belonging to the genus *Dirofilaria* (Spirurida: Onchocercidae) considered to be of great epidemiological relevance. This genus includes 27 valid species and 15 species of which the validity is debated (Dantas-Torres and Otranto, 2013). Culicids transmit these nematodes, and microfilariae are blood-dwelling. Adult parasites are found in different anatomical locations, including the intravascular system (*Dirofilaria immitis*) (Kotani and Powers, 1982) and subcutaneous tissue (*Dirofilaria repens*, *Dirofilaria striata*) (Orihel et al., 1997; Wyatt et al., 2020).

Dirofilaria immitis is North America's most well-known filarioid species infecting companion animals (e.g., dogs, cats, ferrets) (Mosley et al., 2023). Its full life cycle has been extensively studied and involves the ingestion of blood microfilariae by culicid vectors and the development from first-stage to infective third-stage larvae in about 14 days (McCall et al., 2008). In the vertebrate host the parasite requires at least 6 months to reach the adult stage, which is primarily found in the pulmonary arteries and right ventricle (Kotani and Powers, 1982). A variety of wild canids, such as coyotes, red foxes, and wolves, have been reported with infections (Zinck et al., 2021; Sobotyk et al., 2022). In addition, *D. immitis* infections have also been described in captive and wild felids, mustelids, ursids, and even humans (Crum et al., 1978; Papadopoulos et al., 2017; St Jean et al., 2022; Upton et al., 2023). Widely distributed across the United States, this parasite has been reported in all 50 states (Nelson et al., 2005; Little et al., 2021). While the occurrence in domestic dogs and cats is well documented, the infection in wild carnivores is less frequently reported (Kotwa et al., 2019; Sobotyk et al., 2022; Upton et al., 2022).

Dirofilaria striata is another filarioid species infecting wild carnivores in North America. This nematode has been detected in bobcats from Louisiana (Orihel and Ash 1964), in bobcats from South Carolina (Miller

* Corresponding author.
E-mail address: gverocai@cvm.tamu.edu (G.G. Verocai).

https://doi.org/10.1016/j.ijppaw.2024.100954
Received 10 May 2024; Received in revised form 11 June 2024; Accepted 11 June 2024
Available online 12 June 2024

and Harkema 1968), in panthers from Florida (Forrester et al., 1985), and more recently in a domestic cat from Florida (Wyatt et al., 2020). The life cycle of *D. striata* is not entirely known but likely involves infective third-stage larvae (L3) transmitted by culicids (Orihel and Ash 1964). In contrast to *D. immitis*, *D. striata* adults are found in the hosts' subcutaneous tissues (Orihel and Ash 1964). There has been a single report of a zoonotic infection attributed to *D. striata* in the intraorbital tissue of a 9-year-old boy from North Carolina (Orihel and Isbey, 1990).

The role of wild carnivores in the epidemiology of *Dirofilaria* species has been the subject of various studies (Nelson et al., 2003; Magi et al., 2008; Aher et al., 2016). Wild canids can maintain *D. immitis* and act as a source of microfilaria to infect mosquitoes (Bowman and Atkins, 2009). Wildlife infections with *D. immitis* are of particular consideration for maintaining transmission because unlike dogs or cats, wild animals are not covered by any prophylactic program (Brown et al., 2012; Wang et al., 2014). The susceptibility of wild carnivores to *Dirofilaria* infections and the widespread distribution of these animals in North America likely makes them important in the epidemiology of these filarioids. Heartworm infections in wild canid populations, especially coyotes, likely increase the risk of *D. immitis* transmission to domestic animals and humans (Kotwa et al., 2019; Worsley-Tonks et al., 2021; Sobotyk et al., 2022).

Wild carnivores (e.g., coyotes, raccoons) can inhabit anthropized areas close to pet animals and human beings (Gates et al., 2014; Hody and Kays, 2018; Worsley-Tonks et al., 2021). Areas markedly changed by anthropic actions amplify the transmission of vector-borne pathogens, including those of zoonotic concern (Bradley and Altizer, 2007; Brearley et al., 2013; Cardozo et al., 2021). In this study, we used molecular methods to characterize *D. immitis* and *D. striata* in wild carnivores across Texas, United States.

2. Material and methods

2.1. Animals and sampling

We opportunistically tested archival DNA samples (n = 202) extracted from blood clot collected in previous research across 32 counties of Texas, US (Curtis-Robles et al., 2016; Hodo et al., 2020; Salomon et al., 2024, in press). In total, 117 coyotes (*Canis latrans*), 49 raccoons (*Procyon lotor*), 12 gray foxes (*Urocyon cinereoargenteus*), three bobcats (*Lynx rufus*) and one striped skink (*Mephitis mephitis*) were initially included in this study. Additional raccoon (n = 18) and bobcat (n = 2) blood samples were collected during nuisance predator trapping efforts on a private ranch in Atascosa County, Texas in March 2021 and tested as well. Blood samples were collected from the heart or thoracic cavity in the field within 8 h of death. Few of these blood samples were taken from live-capture and released wildlife (IACUC, 2021-0124 D C). From all samples, eight blood samples from Walker County were collected from raccoons each month between May 2021 and May 2022 (Salomon et al., 2024, in press). Three raccoons, one skunk, and one bobcat were sampled through teaching courses in Brazos and Matagorda counties between 2020 and 2023.

2.2. PCR and sequencing

DNA was extracted from blood clots using the Omega E.N.Z.A. Tissue DNA kit (Omega, Biotek, Norcross, GA). The partial cytochrome oxidase c subunit 1 (*COI*) gene (635 bp) of filarial nematodes was amplified using the primers COIintF: 5′-TGA TTG GTG GTT TTG GTA A-3′ and COIintR: 5′-ATA AGT ACG AGT ATC AAT ATC- 3′. These primers generated products of the expected size for eleven species of filarial nematodes (Casiraghi et al., 2001). The cycling conditions included an initial denaturation step at 95 °C for 2 min, followed by 40 cycles at 95 °C for 45 s, 52 °C at 45 s, and 72 °C for 90 s, and a final extension step at 72 °C for 5 min. We used nuclease-free water as a negative control and DNA of *D. immitis* as a positive control. The amplifications were viewed after 1.5% agarose gel electrophoresis in UV transilluminator.

All amplified bands were purified using the E.Z.N.A.® Cycle Pure Kit (OMEGA Bio-Tek Inc., Norcross, GA, USA) according to the manufacturer's instructions and sequenced in both directions using the Sanger method in an ABI-3130 automatic sequencer (Applied Biosystems) at Eurofins Genomics LLC (Houston, TX). Sequences were aligned and compared to homologous sequences of *Dirofilaria* spp. available in the nucleotide sequence database at the National Center of Biotechnology Information (NCBI) (Available online: https://www.ncbi.nlm.nih.gov/genbank/) using ClustalW in MEGA 11 (Clark et al., 2016; Tamura et al., 2021). Phylogenetic analyses for the partial *COI* gene were performed using the Maximum Likelihood Method and a General time Reversible best-fit model with gamma distribution (2000 bootstrap replicates) in MEGA 11 (Tamura et al., 2021). A sequence of *Brugia pahangi* was used as outgroup.

2.3. Data analysis and map creation

The prevalence (%) was calculated for filarial infection along with 95% confidence intervals (95% CI) by the modified Wilson method (https://epitools.ausvet.com.au/ciproportion). Fisher's exact test was used to compare the *D. immitis* positivity between coyotes and raccoons. A 5% significance level was considered. The BioEstat software version 5.3 was used for statistical evaluation (Ayres et al., 2007).

For map creation, cartographic bases in shapefile format were accessed on the United States Census Bureau website. Then, these files were inserted in the QGIS version 3.28.15 software, and the raster layer was generated.

3. Results

Dirofilaria immitis DNA was detected in 11.39% (n = 23/202, 95% CI = 7.71–16.51) of the samples. In particular, 17.95% (n = 21/117, 95% CI = 12.05–25.89) of coyotes and 2.99% (n = 2/67, 95% CI = 0.82–10.95) of raccoons were positive. DNA of *D. striata* was detected in one bobcat (20%; 95% CI = 3.62–62.45). Coyotes had a higher prevalence of *D. immitis* than raccoons (p = 0.0066). All 12 gray foxes and one striped skunk tested negative. Positive animals were distributed in 34.27% (11/32) of counties in which animals were sampled (Fig. 1).

All amplified bands produced a single sequence. In particular, the molecular analyses generated 23 *D. immitis* sequences with a nucleotide identity ranging from 99.85%–100% (99.92% ± 0.08) with other *D. immitis* sequences (ON062406; LC107816). A total of six different *D. immitis* sequences were identified, two of which were shared between coyote and raccoon. Our *D. striata* sequence (PP572465) was 100% similar to the only *COI* sequence available in the GenBank database (MN635457, collected from a domestic cat from Florida) (Wyatt et al., 2020). Our sequences formed a well-supported clade (100% bootstrap support) with other *D. immitis* sequences (Fig. 2). One representative of each unique sequence was deposited in the GenBank database under accession numbers PP566947 – 52.

4. Discussion

This study revealed the presence of two zoonotic species of *Dirofilaria* in three species of wild carnivores. The positivity for *D. immitis* infection in coyotes (17.95%; 21/117) is higher than recently reported in Texas (9.83%; 12/122) (Sobotyk et al., 2022). Overall, a significant variation in prevalence rates has been observed in coyotes from North America, with reports in the last decade varying from 6.5% (Paras et al., 2012) to 37.2% (Aher et al., 2016). Regardless of the factors (e.g., geographic location, climate, population sampled, and animal age) influencing the occurrence of *D. immitis* in coyotes (Brown et al., 2012), reports of this infection in the southern United States where temperature is more suitable for mosquito development have increased over the last years (Gates et al., 2014; Aher et al., 2016; Sobotyk et al., 2022).

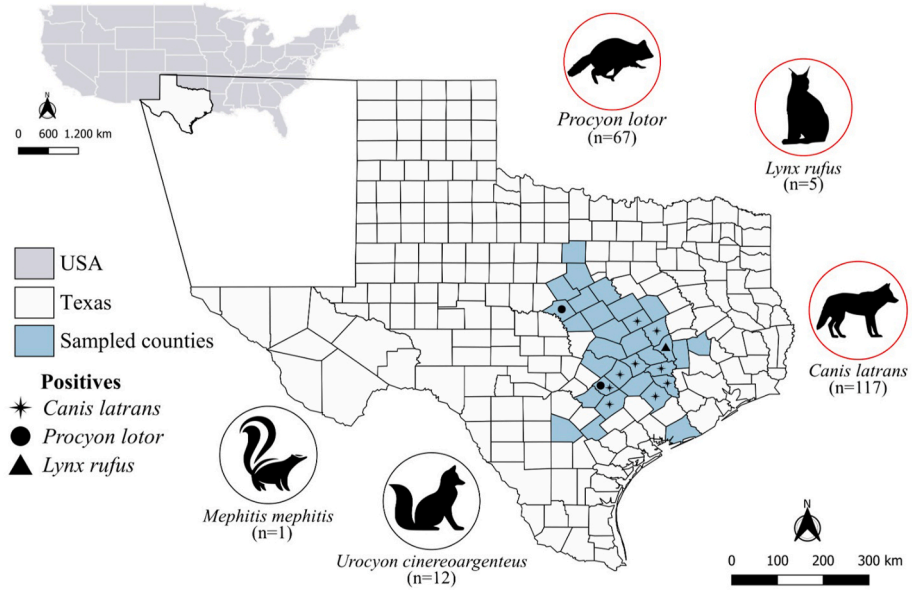

Fig. 1. Map of Texas, US indicating the origin and number of animals sampled by species. Counties of Texas from which samples originated are in blue. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

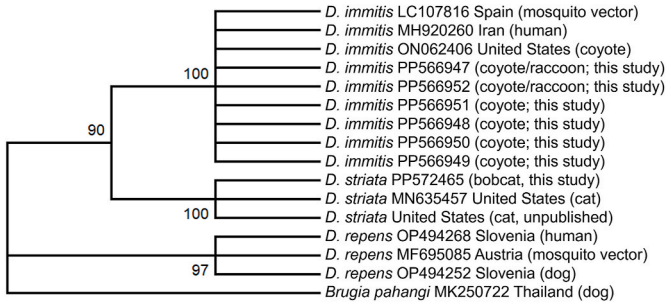

Fig. 2. Phylogenetic tree created using a maximum likelihood method (2000 bootstrap replicates) showing the relationship of identified *Dirofilaria immitis* and one isolate of *Dirofilaria striata*. *Brugia pahangi* sequence was used as outgroup.

The apparent absence of *D. immitis* in gray foxes could be attributed to the limited opportunistic sample size available in the current study. There have been several reports of *D. immitis* in gray foxes across areas of the U.S. and Mexico, often in low prevalence (Simmons et al., 1980; Carlson and Nielsen, 1983; Hernández-Camacho et al., 2016).

In this study, we detected *D. immitis* DNA in 2.99% (2/67) of raccoons. In North America, *Dirofilaria* infections in raccoons are primarily attributed to *Dirofilaria tenuis* (Sauerman and Nayar, 1985; Izasa and Courtney, 1988; Telford and Forrester, 1991; Richardson et al., 1992; Pung et al., 1996). The absence of *D. tenuis* DNA sequences in GenBank is a limitation for studies in which blood samples of raccoons are molecularly assessed. Hence, molecular characterization of *D. tenuis* genes is urgently needed, especially because it is the third most common *Dirofilaria* species reported in humans (Perles et al., 2024). A single juvenile male raccoon was previously reported to be naturally infected by two immature female *D. immitis* in Parramore Island, Virginia (Snyder et al., 1989).; In addition, a previous experimental study had demonstrated that raccoons could not support the development of *D. immitis* (Christensen and Shelton, 1978); however only two adult female raccoons were used in this study. While generally considered an aberrant host with no significant role in the epidemiology of *D. immitis*, the detection of parasite DNA in blood suggests the presence of circulating microfilariae, and hence, a patent infection (Negron et al., 2022; Sobotyk et al., 2022). Nevertheless, the role of raccoons as a susceptible, relevant reservoir host for *D. immitis* remains questionable and may deserve further investigation.

Dirofilaria striata, a scarcely studied filarioid nematode of felids, was found in a single bobcat (1/5). This positive bobcat was captured within a neighborhood illustrating their epidemiological importance as reservoir to other hosts, including domestic cats. This parasite has been reported in bobcats in Louisiana (Orihel and Ash, 1964), South Carolina (Miller and Harkema, 1968), Florida panthers (*Puma concolor*) (Forrester et al., 1985; Lamm et al., 1997), and dogs in Florida (Courtney et al., 1985). More recently, the detection of this parasite in a cat from Florida provided the first molecular data based on the partial sequence of the *COI* gene (Wyatt et al., 2020). The molecular characterization of novel *D. striata* isolates across its range is paramount for establishing a robust database for future comparisons among filarial populations.

Despite being underdiagnosed in humans, the zoonotic role of *D. immitis* is known worldwide (Theis, 2005; Fontes-Sousa et al., 2019; Oshima, 2023; Perles et al., 2024). Classical infections in humans are characterized by nodules on the lungs with minimal pathogenic relevance (Hirano et al., 2002; Malik et al., 2016; Palicelli et al., 2022). However, erratic ocular parasitism by *D. immitis* may pose additional risk to human health (Somsap et al., 2021; Aykur et al., 2021). Additionally, human infection by *D. striata* was reported in the intraorbital tissue of a 6-year-old boy living in Buncombe County, North Carolina (Orihel and Isbey, 1990).

The presence of both *D. immitis* and *D. striata* in wild carnivores, especially those adapted to live in areas inhabited by domestic animals and humans, is essential for understanding dirofilariosis epidemiology. While in endemic areas, chemoprophylactic treatments impact the overall prevalence of the infection in dogs and cats (Labarthe et al., 2015; Brianti et al., 2023; Mosley et al., 2023). However, the lack of measures to prevent infection in coyotes, raccoons and bobcats may negatively impact any prevention or eradication program, like has been suggested in the Linosa Island, in Italy, where, most likely, the presence of feral cats could impact the eradication program (Brianti et al., 2023). Finally, data from this study indicate that *D. immitis* infection in raccoons is likely more common than expected and reinforces the importance of monitoring both coyotes and raccoons in heartworm endemic areas. Additionally, it provides additional molecular data on *D. striata*, a little known filarioid of felids.

CRediT authorship contribution statement

Rafael A.N. Ramos: Writing – original draft, Methodology, Data curation, Conceptualization. **Hassan Hakimi:** Writing – review & editing, Methodology, Data curation. **Jordan Salomon:** Writing – review & editing, Methodology, Data curation. **Rachel E. Busselman:** Writing – review & editing, Methodology, Data curation. **Rachel Curtis-Robles:** Writing – review & editing, Methodology, Data curation. **Carolyn L. Hodo:** Writing – review & editing, Methodology, Data curation. **Sarah A. Hamer:** Writing – review & editing, Supervision, Funding acquisition, Data curation, Conceptualization. **Guilherme G. Verocai:** Writing – review & editing, Supervision, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

None.

Acknowledgements

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors. Authors thank Samuel Sousa Silva (Federal University of the Agreste of Pernambuco, Brazil) for preparing Fig. 1.

References

Aher, A.M., Caudill, D., Caudill, G., Butryn, R.S., Wolf, D., Fox, M., Blake, D.P., Cunningham, M.W., 2016. Prevalence, genetic analyses, and risk factors associated with heartworm (*Dirofilaria immitis*) in wild coyotes (*Canis latrans*) from Florida, USA. J. Wildl. Dis. 52, 785–792. http://doi:10.7589/2015-09-223.

Aykur, M., Yağcı, A., Simşek, S., Palamar, M., Yaman, B., Korkmaz, M., Dagci, H., 2021. First time identification of subconjunctival *Dirofilaria immitis* in Turkey: giant episcleral granuloma mimicking scleritis. Parasitol. Res. 120, 3909–3914. http://doi:10.1007/s00436-021-07317-2.

Ayres, M., Ayres, M.Jr., Ayres, D.L., Santos, A.A., 2007. BioEstat 5.0: Statistical Applications in the Areas of Biological Sciences and Medical. Institute for Sustainable Development Mamirauá, Amazonas.

Bowman, D.D., Atkins, C.E., 2009. Heartworm biology, treatment, and control. Vet. Clin. Small Anim. 39, 1127–1158. http://doi:10.1016/j.cvsm.2009.06.003.

Bradley, C.A., Altizer, S., 2007. Urbanization and the ecology of wildlife diseases. Trends Ecol. Evol. 22, 95–102. http://doi:10.1016/j.tree.2006.11.001.

Brearley, G., Rhodes, J., Bradley, A., Baxter, G., Seabrook, L., Lunney, D., Liu, Y., McAlpine, C., 2013. Wildlife disease prevalence in human-modified landscapes. Biol. Rev. Camb. Phil. Soc. 88, 427–442. http://doi:10.1111/brv.12009.

Brianti, E., Napoli, E., De Benedetto, G., Venco, L., Mendoza-Roldan, J.A., Basile, A., Bezerra-Santos, M.A., Drake, J., Schaper, R., Otranto, D., 2023. Elimination of *Dirofilaria immitis* infection in dogs, Linosa Island, Italy, 2020-2022. Emerg. Infect. Dis. 29, 1559–1565. http://doi:10.3201/eid2908.221910.

Brown, H.E., Harrington, L.C., Kaufman, P.E., McKay, T., Bowman, D.D., Nelson, C.T., Wang, D., Lund, R., 2012. Key factors influencing canine heartworm, *Dirofilaria immitis*, in the United States. Parasites Vectors 5, 245. http://doi:10.1186/1756-3305-5-245.

Carlson, B.L., Nielsen, S.W., 1983. *Dirofilaria immitis* infection in a gray fox. J. Am. Vet. Med. Assoc. 183, 1275–1276.

Cardozo, M., Fiad, F.G., Crocco, L.B., Gorla, D.E., 2021. Effect of habitat fragmentation on rural house invasion by sylvatic triatomines: a multiple landscape-scale approach. PLoS Neglected Trop. Dis. 15, e0009579. http://doi:10.1371/journal.pntd.0009579.

Casiraghi, M., Anderson, T.J.C., Bandi, C., Bazzocchi, C., Genchi, C., 2001. A phylogenetic analysis of filarial nematodes: comparison with the phylogeny of *Wolbachia* endosymbionts. Parasitology 122, 93–103. https://doi.org/10.1017/S0031182000007149.

Christensen, B.M., Shelton, M.E., 1978. Laboratory observations on the insusceptibility of raccoons to *Dirofilaria immitis*. J. Wildl. Dis. 14, 22–23. http://doi:10.7589/0090-3558-14.1.22.

Clark, K., Karsch-Mizrachi, I., Lipman, D.J., Ostell, J., Sayers, E.W., 2016. GenBank. Nucleic. Acids Res. 44, 67–72. http://doi:10.1093/nar/gkv1276.

Courtney, C.H., Sundlof, S.F., Lane, T.J., 1985. Impact of filariasis on the racing greyhound. J. Am. Anim. Hosp. Assoc. 21, 421–425.

Crum, J.M., Nettles, V.F., Davidson, W.R., 1978. Studies on endoparasites of the Black bear (*Ursus americanus*) in the southeastern United States. J. Wildl. Dis. 14, 178–186. https://doi.org/10.7589/0090-3558-14.2.178.

Curtis-Robles, R., Lewis, B.C., Hamer, S.A., 2016. High *Trypanosoma cruzi* infection prevalence associated with minimal cardiac pathology among wild carnivores in central Texas. Int. J. Parasitol. Parasites Wildl. 5, 117–123. http://doi:10.1016/j.ijppaw.2016.04.001.

Dantas-Torres, F., Otranto, D., 2013. Dirofilariosis in the Americas: a more virulent *Dirofilaria immitis*? Parasites Vectors 6, 1–9. https://doi.org/10.1186/1756-3305-6-288.

Fontes-Sousa, A.P., Silvestre-Ferreira, A.C., Carretón, E., Esteves-Guimarães, J., Maia-Rocha, C., Oliveira, P., Lobo, L., Morchón, R., Araújo, F., Simón, F., Montoya-Alonso, J.A., 2019. Exposure of humans to the zoonotic nematode *Dirofilaria immitis* in Northern Portugal. Epidemiol. Infect. 147, e282. http://doi:10.1017/S0950268819001687.

Forrester, D.J., Conti, J.A., Belden, R.C., 1985. Parasites of the Florida panther (*Felis concolor coryi*). Proc. Helm. Soc. Wash. 52, 95–97.

Gates, M., Gerhold, R.W., Wilkes, R.P., Gulsby, W.D., Maestas, L., Rosypal, A., Miller, K. V., Miller, D.L., 2014. Parasitology, virology, and serology of free-ranging coyotes (*Canis latrans*) from central Georgia, USA. J. Wildl. Dis. 50, 896–901. http://doi:10.7589/2013-10-283.

Gruntmeir, J., Kelly, M., Ramos, R.A.N., Verocai, G.G., 2023. Cutaneous filarioid nematodes of dogs in the United States: are they emerging, neglected, or underdiagnosed parasites? Front. Vet. Sci. 10, 1128611. http://doi:10.3389/fvets.2023.1128611.

Hernández-Camacho, N., Pineda-López, R.F., de Jesús Guerrero-Carrillo, M., Cantó-Alarcón, G.J., Jones, R.W., Moreno-Pérez, M.A., Mosqueda-Gualito, J.J., Zamora-Ledesma, S., Camacho-Macías, B., 2016. Gray fox (*Urocyon cinereoargenteus*) parasite diversity in central Mexico. Int. J. Parasitol. Parasites Wildl. 5, 207–210. http://doi:10.1016/j.ijppaw.2016.06.003.

Hirano, H., Kizaki, T., Sashikata, T., Matsumura, T., 2002. Pulmonary dirofilariasis–clinicopathological study. Kobe J. Med. Sci. 48, 79–86.

Hodo, C.L., Bañuelos, R.M., Edwards, E.E., Wozniak, E.J., Hamer, S.A., 2020. Pathology and discrete typing unit associations of *Trypanosoma cruzi* infection in coyotes (*Canis latrans*) and raccoons (*Procyon lotor*) of Texas, USA. J. Wildl. Dis. 56, 134–144. http://doi:10.7589/2019-03-071.

Hody, J.W., Kays, R., 2018. Mapping the expansion of coyotes (*Canis latrans*) across North and Central America. ZooKeys 759, 81–97. http://doi:10.3897/zookeys.759.15149.

Isaza, R., Courtney, C.H., 1988. Possible association between *Dirofilaria tenuis* infections in humans and its prevalence in raccoons in Florida. J. Parasitol. 74, 189–190.

Kotani, T., Powers, K.G., 1982. Developmental stages of *Dirofilaria immitis* in the dog. Am. J. Vet. Res. 43, 2199–2206.

Kotwa, J.D., Jardine, C.M., Berke, O., Pearl, D.L., Mercer, N.J., Peregrine, A.S., 2019. Prevalence and distribution of *Dirofilaria immitis* infection in wild canids in southern Ontario. Vet. Parasitol. Reg. Stud. Reports. 18, 100349. http://doi:10.1016/j.vprsr.2019.100349.

Labarthe, N.V., Willi, L.M., Paiva, J.P., Miranda, M.G., Zoreck, K., Almeida, F.M., 2015. Chemoprophylaxis of *Dirofilaria immitis* (Leidy 1856) infection at a high challenge environment. Parasites Vectors 12, 523. http://doi:10.1186/s13071-015-1141-6.

Lamm, M.G., Roelke, M.E., Greiner, E.C., Streible, C.K., 1997. Microfilariae in the free-ranging Florida panther (*Felis concolor coryi*). J. Helm. Soc. Wash. 64, 137–141.

Little, S., Braff, J., Place, J., Buch, J., Dewage, B.G., Knupp, A., Beall, M., 2021. Canine infection with *Dirofilaria immitis, Borrelia burgdorferi, anaplasma* spp., and *ehrlichia* spp. in the United States, 2013-2019. Parasites Vectors 14, 10. http://doi:10.1186/s13071-020-04514-3.

McCall, J.W., Genchi, C., Kramer, L.H., Guerrero, J., Venco, L., 2008. Heartworm disease in animals and humans. Adv. Parasitol. 66, 193–285. http://doi:10.1016/S0065-308X(08)00204-2.

Magi, M., Calderini, P., Gabrielli, S., Dell'Omodarme, M., Macchioni, F., Prati, M.C., Cancrini, G., 2008. *Vulpes vulpes*: a possible wild reservoir for zoonotic filariae. Vector Borne Zoonotic. Dis 8, 249–252. http://doi:10.1089/vbz.2007.0207.

Malik, D., Amaraneni, A., Singh, S., Roach, R., 2016. Man's best friend: how humans can develop *Dirofilaria immitis* infections. IDCases 24, 43–45. http://doi:10.1016/j.idcr.2016.03.003.

Miller, G.C., Harkema, R., 1968. Helminths of some wild mammals in the southeastern United States. Proc Helminthol Soc Wash 35, 118–125.

Mosley, I.A., Zecca, I.B., Tyagi, N., Harvey, T.V., Hamer, S.A., Verocai, G.G., 2023. Occurrence of *Dirofilaria immitis* infection in shelter cats in the lower Rio Grande Valley region in South Texas, United States, using integrated diagnostic approaches. Vet. Parasitol. Reg. Stud. Rep. 41, 100871 https://doi.org/10.1016/j.vprsr.2023.100871.

Negron, V., Saleh, M.N., Sobotyk, C., Luksovsky, J.L., Harvey, T.V., Verocai, G.G., 2022. Probe-based qPCR as an alternative to modified Knott's test when screening dogs for heartworm (*Dirofilaria immitis*) infection in combination with antigen detection tests. Parasites Vectors 15, 306. https://doi.org/10.1186/s13071-022-05372-x.

Nelson, T.A., Gregory, D.G., Laursen, J.R., 2003. Canine heartworms in coyotes in Illinois. J. Wildl. Dis. 39, 593–599. http://doi:10.7589/0090-3558-39.3.593.

Nelson, C.T., McCall, J.W., Rubin, S.B., Buzhardt, L.F., Dorion, D.W., Graham, W., Longhofer, S.L., Guerrero, J., Robertson-Plouch, C., Paul, A., 2005. Guidelines for the diagnosis, prevention and management of heartworm (*Dirofilaria immitis*) infection in dogs. Vet. Parasitol. 133, 255–266. http://doi:10.1016/j.vetpar.2005.07.008.

Orihel, T.C., Ash, L.R., 1964. Occurrence of *Dirofilaria striata* in the bobcat (*Lynx rufus*) in Louisiana with observations on its larval development. J. Parasitol. 50, 590–591.

Orihel, T.C., Helentjaris, D., Alger, J., 1997. Subcutaneous dirofilariasis: single inoculum, multiple worms. Am. J. Trop. Med. Hyg. 56, 452–455. http://doi:10.4269/ajtmh.1997.56.452.

Orihel, T.C., Isbey, E.K., 1990. *Dirofilaria striata* infection in a North Carolina child. Am. J. Trop. Med. Hyg. 42, 124–126.

Oshima, K., 2023. Clinical characteristics of human pulmonary dirofilariasis in Japan: an uncommon differential diagnosis of a solitary pulmonary nodule. Jpn. J. Infect. Dis. 76, 310–313. http://doi:10.7883/yoken.JJID.2022.617.

Otranto, D., Deplazes, P., 2019. Zoonotic nematodes of wild carnivores. Int. J. Parasitol. Parasites Wildl. 9, 370–383. http://doi:10.1016/j.ijppaw.2018.12.011.

Palicelli, A., Veggiani, C., Rivasi, F., Gustinelli, A., Boldorini, R., 2022. Human Pulmonary Dirofilariasis due to *Dirofilaria immitis*: the first Italian case confirmed by polymerase chain reaction analysis, with a systematic literature review. Life 12, 1584. http://doi:10.3390/life12101584.

Papadopoulos, E., Komnenou, A., Poutachides, T., Heikkinen, P., Oksanen, A., Karamanlidis, A.A., 2017. Detection of *Dirofilaria immitis* in a brown bear (*Ursus arctos*) in Greece. Helminthologia 54, 257–261. https://doi.org/10.1515/helm-2017-0033.

Paras, K.L., Little, S.E., Reichard, M.V., Reiskind, M.H., 2012. Detection of *Dirofilaria immitis* and *Ehrlichia* species in coyotes (*Canis latrans*), from rural Oklahoma and Texas. Vector Borne Zoonotic Dis. 12, 619–621. http://doi:10.1089/vbz.2011.0815.

Perles, L., Dantas-Torres, F., Krücken, J., Morchón, R., Walochnik, J., Otranto, D., 2024. Zoonotic dirofilariases: one, no one, or more than one parasite. Trends Parasitol. 40, 257–270. http://doi:10.1016/j.pt.2023.12.007.

Pung, O.J., Davis, P.H., Richardson, D.J., 1996. Filariae of raccoons from southeast Georgia. J. Parasitol. 82, 849–851.

Richardson, D.J., Owen, W.B., Snyder, D.E., 1992. Helminth parasites of the raccoon (*Procyon lotor*) from north-central Arkansas. J. Parasitol. 78, 163–166.

Sauerman, D.M.Jr., Nayar, J.K., 1985. Prevalence of presumed *Dirofilaria tenuis* microfilariae in raccoons near Vero Beach, Florida. J. Parasitol. 71, 130–132.

Simmons, J.M., Nicholson, W.S., Hill, E.P., Briggs, D.B., 1980. Occurrence of (*Dirofilaria immitis*) in gray fox (*Urocyon cinereoargenteus*) in Alabama and Georgia. J. Wildl. Dis. 16, 225–228. http://doi:10.7589/0090-3558-16.2.225.

Snyder, D.E., Hamir, A.N., Hanlon, C.A., Rupprecht, C.E., 1989. *Dirofilaria immitis* in a raccoon (*Procyon lotor*). J. Wildl. Dis. 25, 130–131. http://doi:10.7589/0090-3558-25.1.130.

Sobotyk, C., Nguyen, N., Negrón, V., Varner, A., Saleh, M.N., Hilton, C., Tomeček, J.M., Esteve-Gasent, M.D., Verocai, G.G., 2022. Detection of *Dirofilaria immitis* via integrated serological and molecular analyses in coyotes from Texas, United States. Int. J. Parasitol. Parasites Wildl. 18, 20–24. http://doi:10.1016/j.ijppaw.2022.03.012.

Somsap, Y., Boonroumkaew, P., Somsap, A., Rodpai, R., Sadaow, L., Sanpool, O., Maleewong, W., Intapan, P.M., 2021. Ocular dirofilariasis case in Thailand confirmed by molecular analysis to be caused by *Dirofilaria immitis*. Am. J. Trop. Med. Hyg. 106, 204–207. http://doi:10.4269/ajtmh.21-0764.

St Jean, S.C., Bryan, L.K., Sobotyk, C., Verocai, G.G., 2022. Pathology in Practice: aberrant intra-cranial *Dirofilaria immitis* migration with cerebral and cerebellar trauma in a Fennec Fox (*Vulpes zerda*). J. Am. Vet. Med. Assoc. 259, 1–3. https://doi.org/10.2460/javma.20.08.0469.

Tamura, K., Stecher, G., Kumar, S., 2021. MEGA11: molecular evolutionary genetics analysis version 11. Mol. Biol. Evol. 38, 3022–3027. http://doi:10.1093/molbev/msab120.

Telford, S.R.Jr., Forrester, D.J., 1991. Hemoparasites of raccoons (*Procyon lotor*) in Florida. J. Wildl. Dis. 27, 486–490. http://doi:10.7589/0090-3558-27.3.486.

Theis, J.H., 2005. Public health aspects of dirofilariasis in the United States. Vet. Parasitol. 133, 157–180. http://doi:10.1016/j.vetpar.2005.04.007.

Upton, K.E., Sobotyk, C., Edwards, E.E., Verocai, G.G., 2022. *Dirofilaria immitis* in an Asian small-clawed otter (*Aonyx cinereus*) from southeastern Louisiana, United States. Vet. Parasitol. Reg. Stud. Rep. 29, 100703 https://doi.org/10.1016/j.vprsr.2022.100703.

Upton, K.E., Budke, C.M., Verocai, G.G., 2023. Heartworm (*Dirofilaria immitis*) in carnivores kept in zoos in Texas, USA: risk perception, practices, and antigen detection. Parasites Vectors 16, 150. http://doi:10.1186/s13071-023-05750-z.

Wang, D., Bowman, D.D., Brown, H.E., Harrington, L.C., Kaufman, P.E., McKay, T., Nelson, C.T., Sharp, J.L., Lund, R., 2014. Factors influencing U.S. canine heartworm (*Dirofilaria immitis*) prevalence. Parasites Vectors 7, 264. http://doi:10.1186/1756-3305-7-264.

Worsley-Tonks, K.E.L., Gehrt, S.D., Anchor, C., Escobar, L.E., Craft, M.E., 2021. Infection risk varies within urbanized landscapes: the case of coyotes and heartworm. Parasites Vectors 14, 464. http://doi:10.1186/s13071-021-04958-1.

Wyatt, D., Santoro, D., Deabold, K., Gruntmeir, J., Childress, A., Craft, W.F., Walden, H.D.S., Wellehan, J.F.X., 2020. Subcutaneous nodules and dermatitis associated with non-immitis non-repens dirofilariosis morphologically consistent with *Dirofilaria striata* in a 2-year-old male domestic cat in Florida, USA. Vet. Q. 40, 215–222. http://doi:10.1080/01652176.2020.1814972.

Zinck, C.B., Priest, J.M., Shutler, D., Boudreau, M., Lloyd, V.K., 2021. Detection of *Borrelia* spp., *Ehrlichia canis, Anaplasma phagocytophilum*, and *Dirofilaria immitis* in eastern coyotes (*Canis latrans*) in nova scotia, Canada. J. Wildl. Dis. 57, 678–682. http://doi:10.7589/JWD-D-20-00188.