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Research Note

A case of natural infection with *Dirofilaria immitis* in a coati (*Nasua narica*) from Mexico

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Summary

This paper aims to describe the natural infection with *Dirofilaria immitis* in *Nasua narica* (white-nosed coati) from Yucatán, Mexico. Two carcasses of *N. narica* were collected on a highway that crosses through a dense forest with patches used for agriculture and livestock activities. We performed necropsies, and two female adult nematode parasites from the heart of one specimen were collected and preserved for their molecular identification using a conventional PCR directed at a fragment of the small subunit (18S) ribosomal RNA (*18S*-rRNA) gene. Bioinformatic analysis showed a similarity of 99 % with three sequences from *D. immitis* (two from Japan). Additionally, we performed a phylogenetic tree with the recovered sequence. All these analyses showed that *D. immitis* is present in *N. narica* from Mexico. The transmission of *D. immitis* toward populations of *Nasua* sp. may be due to indirect and accidental contact with domestic dogs or wild canids that share the same environment.
Keywords: Wildlife; nematode; infection; carnivores

Introduction

It is known that wildlife is a significant element in maintaining and distributing the enzootic transmission cycles of numerous pathogens of importance to public or animal health. Wild animals from the Yucatán Peninsula, Mexico, such as rodents, bats, and some medium-sized mammals, have been registered as hosts of several pathogens, such as *Rickettsia* sp. (Panti-May *et al*., 2015), *Leptospira* sp. (Torres-Castro *et al*., 2020), *Trypanosoma cruzi* (Torres-Castro *et al*., 2021), *Toxoplasma gondii* (Torres-Castro *et al*., 2019), *Leishmania* sp. (Sosa-Bibiano *et al*., 2022), and different nematode species (Panti-May *et al*., 2021). All these findings reflect the need to continue with epidemiological studies that help to understand the role of wildlife in the life cycles of pathogens with zoonotic potential and to improve the knowledge about the health of their populations.

Dirofilaria immitis (Spirurida: Onchocercidae) is a parasitic nematode species widely distributed in areas with tropical and subtropical climates, such as the Yucatán Peninsula. It is colloquially known as "heartworm" and affects several wild and domestic animals belonging to different orders, such as Artiodactyla, Carnivora, Edentata, Lagomorpha, Perissodactyla, Primates, and Rodentia; however, canids and felids are its primary hosts (Simón *et al*., 2012; Penezić *et al*., 2014).

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The disease caused by *D. immitis*, known as dirofilariasis, is recognized as a significant animal health problem due to its high prevalence and incidence rates in domiciliated dogs from endemic areas (Bolio-González *et al*., 2007; Caro-González *et al*., 2020). Likewise, in some countries in the Americas and Europe, clinical cases have been reported in people with lung conditions, so pulmonary dirofilariasis is cataloged as a disease potentially emerging due to the wide distribution of the biological vectors. Mosquitoes of the family Culicidae (Dantas-Torres & Otranto, 2013; Penezić *et al*., 2014) are the primary vectors and acquire infective microfilariae when feeding on the blood of infected hosts (Simón *et al*., 2012). Several mosquito species from the genera *Aedes* (*Ae*.), *Anopheles*, *Culex*, and *Ochlerotatus* have been described as probable vectors of *D. immitis* in endemic areas of the Americas (Dantas-Torres & Otranto, 2013).

There is evidence of *D. immitis* natural infection in wild animals (Simón *et al*., 2012; Penezić *et al*., 2014), including coatis (*Nasua* sp.) in South America (Moraes *et al*., 2017; Figuerêdo Duarte Moraes *et al*., 2022). However, epidemiological information on the involvement of this meso-mammal in the transmission cycle of *D. immitis* is scarce and limited worldwide (Vezzani *et al*., 2006), and in Mexico, the information is minimal and only in a morphological way (Caballero-Caballero, 1948). So, this study aims to report the natural infection with *D. immitis* in the white-nosed coati *Nasua narica* (colloquially known as "coati") from Yucatán, Mexico.

Materials and Methods

We collected two carcasses (20°46'6.86"N-88°49'32.30"W and 20°45'44.59"N-88°47'9.35"W) of *N. narica* adult males on the Mérida-Cancún highway (Fig. 1A). Both were evaluated *in situ* to determine the presence of postmortem changes such as cadaveric rigidity, putrefaction, tympanism, etc., and deposited in a portable fridge with refrigerants for transport to the Faculty of Veterinary Medicine and Zootechnics of the Autonomous University of Yucatán (UADY), Mérida, Mexico, for processing. Due to the extensive damage in the carcasses (we suspected that the animals were impacted by vehicles) could not be prepared by conventional taxidermy to enter a mammal collection and obtain the corresponding record.

In the macroscopic exploration of the heart in one specimen, two adult nematode parasites of the right ventricle (Fig. 1B) were collected and stored in 1.5 mL microcentrifuge tubes (Pirouet®, United States of America [USA]) soaked in 96 % alcohol for their subsequent identification by bioinformatics and phylogenetic analyses. Severe hemorrhages were observed in the heart (atria and ventricles); however, these findings should be taken carefully due to the probable cause of death.

Genomic DNA was extracted from an adult female nematode by making a diagonal incision in the midportion of the body. The specimen was placed in 500 µL of a 10 % Chelex-100® (Bio-Rad®, USA) chelating resin solution, to which 20 µL of proteinase were

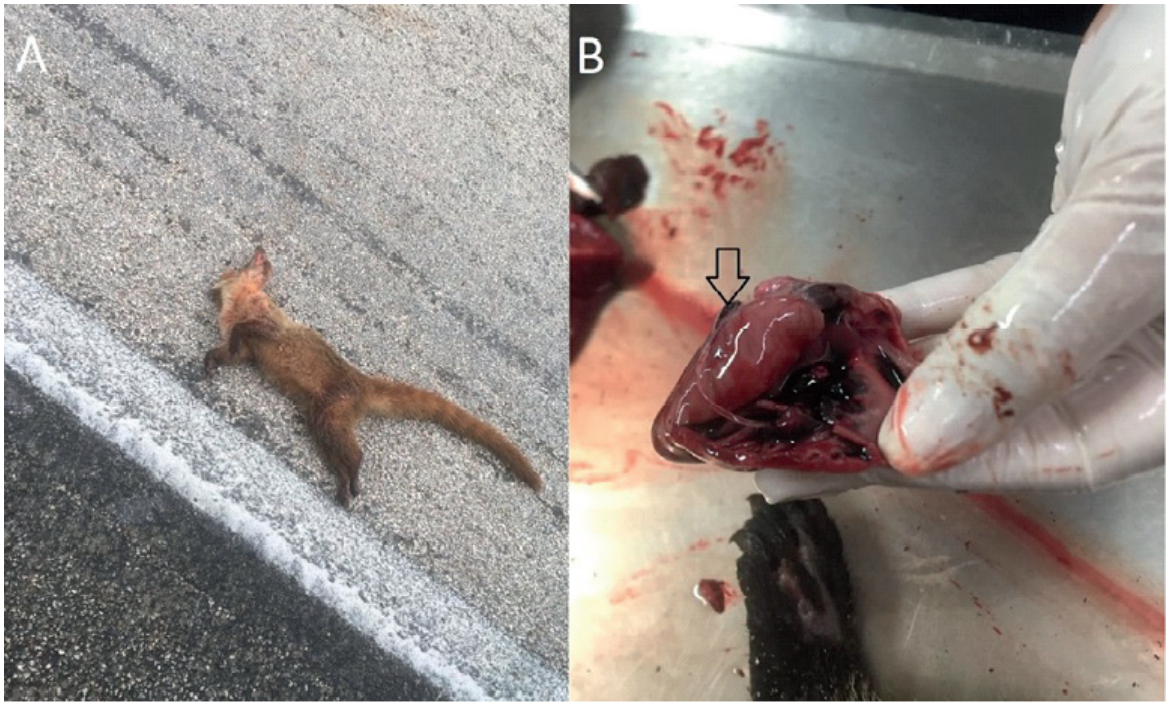

Fig. 1. A) Image of one of the specimens (*Nasua narica*) collected on the Mérida-Cancún highway, Mexico. B) The aspect of the nematodes (arrow) collected in the heart. It can be seen that they are worms with elongated, cylindrical, and non-segmented bodies with bilateral symmetry.

added (Aguilar-Domínguez *et al*., 2019). The sample was incubated at 56° C for 12 h, and the temperature was raised to 100° C for 15 minutes to eliminate the potential PCR inhibitors and increase the degradation of the proteins. The supernatant with the DNA was placed in a new tube and frozen at -20° C until its later use.

The purity of the extracted DNA was measured by the absorbance method with a NanoDrop™ 3300 (Thermo Fisher®, USA). As an endogenous control and for the molecular identification of the nematode species, a fragment of 600 bases of pairs (bp) of the small subunit (18S) ribosomal RNA (*18S-r*RNA) gene was amplified using the oligonucleotides 18S 965 (5'-GGCGATCAGATACCGCCCTAGTT-3') and 18S 1573R (5'-TACAAAGGGCAGGGACGTAAT-3') (Mullin *et al*., 2005). The reaction mixture was done in a final volume of 25 µL, using 12.5 µL of GoTaq® Green Master Mix 2X (Promega Corporation®, USA), 2 µL of primers (2 µM, 1 µL each), 8.5 µL nuclease-free water, and 500 ng DNA (8 µL). We used *Ascaris lumbricoides* DNA as positive control and nuclease-free water as a negative control. The PCR products were analyzed on 1.5 % agarose gels using the Gene Ruler™ Ladder 100 bp DNA marker (Thermo Fisher Scientific®, USA). The gels were stained with SmartGlow™ LD® and visualized in a transilluminator (UVI Tec©; United Kingdom) with an integrated camera for recording results.

The PCR product was purified and sequenced at Macrogen Inc., Korea. Once the partial sequence was obtained, it was compared with those sequences available in GenBank, using the Basic Local Alignment Search Tool (BLAST-n, http://www.ncbi.nlm.nih.gov). Global alignments were performed using the Clustal W algorithm, and the nucleotide substitution model was selected based on the lowest Bayesian Information Criterion (BIC). To identify the phylogenetic position of the nematode, a Maximum Likelihood phylogenetic tree was generated with a support value of 10,000 Bootstrap iterations in IQ-TREE (http://www.iqtree.org/).

Unfortunately, due to the conservation method, both nematode parasites suffered severe damage to their structure, and it was not possible to use them for photomicrography.

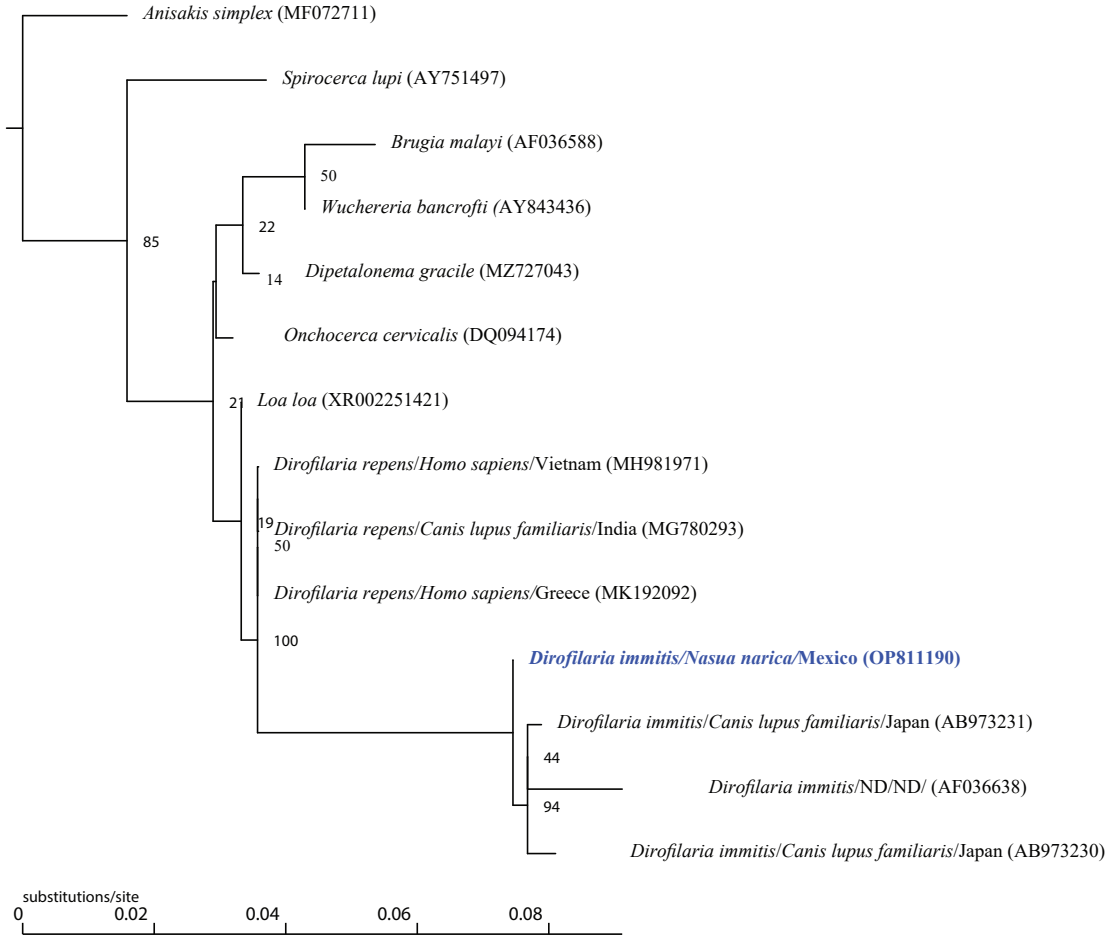

Fig. 2. Phylogenetic reconstruction based on a partial fragment of the *18S-r*RNA gene of several nematode species inferred by Maximum Likelihood and based on the Kimura two parameter Model (K2) with Gamma distribution (+G). Bootstrap values are indicated at the nodes. The scale bar at the bottom represents the degree of divergence.

Ethical Approval and/or Informed Consent

This article does not contain any studies with human participants or live animals.

Results and Discussion

We recovered a sequence of 516 bp of the *18S-r*RNA gene, which exhibited a coverage of 100 % and similarity of 99 % (514/516 bp) with two sequences of *D. immitis* collected from dogs in Japan in 2010 (GenBank accession numbers: AB973230 and AB973231).
The phylogenetic analysis grouped the sequence generated in this study with the only three sequences of *D. immitis* available and deposited in GenBank in a monophyletic group with a support value of 94, which had *Dirofilaria repens* as a sister group with a support value of 100. The sequence generated in this study was deposited in GenBank under Accession number OP811190 (Fig. 2).
The role of wildlife in the maintenance and spread of *D. immitis* is a matter of growing speculation because it is not understood whether wildlife may act as reservoirs, sentinels, or an accidental hosts and which species are significant in the sylvatic cycle (Moroni *et al*., 2020). The molecular, bioinformatics and phylogenetic analysis performed with the sequence obtained from the amplified fragment of the *18S-rRNA* gene show the natural infection with *D. immitis* in *N. narica* from Yucatán, Mexico; therefore, these mammals participate in the epidemiological cycle of the parasite in this region.
As far as we know, this is the first molecular description of the *D. immitis* natural infection in *N. narica* from Mexico and North America. On an international level, the records of natural infection with *D. immitis* in *Nasua* sp. have been described in Argentina (Mazza, 1926; Mancebo *et al*., 1992) and Brazil (Figuerêdo Duarte Moraes *et al*., 2022). Additionally, in the Americas, the generated sequence in this study is the first sequence of *D. immitis* for this mammal host.
In Yucatán, *D. immitis* infects actively and frequently domiciled dogs from urban and rural sites (Bolio-González *et al*., 2007; Caro-González *et al*., 2020). This epidemiological aspect is relevant because the natural infection in domiciliated dogs has been related to *D. immitis* transmission towards medium-sized carnivore mammals such as coatis (*Nasua* sp.) since, eventually, both populations of animals have accidental contact. In addition, it has been suggested that dogs and coatis are involved in the same *D. immitis* transmission cycle, generated and maintained by mosquito vectors that circulate in the environment they share (Moraes *et al*., 2017; Figuerêdo Duarte Moraes *et al*., 2022).
To the best of our knowledge, this is the first study where *N. narica* carcasses are analyzed for the detection of heart nematodes, particularly *D. immitis*. The few previous reports, where necropsies have been performed on other procyonids species, show a low abundance of this nematode species, with two specimens recovered on *Procyon lotor* in the southeastern USA (Snyder *et al*., 1989). In a natural transmission experiment study performed on *P. lotor* it was possible to identify that nematodes did not develop in the two specimens exposed to infected mosquitoes (Christensen & Shelton, 1978). In contrast, dogs used as controls demonstrated the presence of a large number of adult nematodes. Necropsies performed on dogs in endemic areas show the presence of a high number of adult nematodes (Santoro *et al*., 2019). Therefore the role of procyonids as incidental hosts of *D. immitis* should be evaluated.
The present infection case was registered in an individual found on a highway that crosses through a dense forest; however, areas of variable extension destined for livestock and agriculture can be observed. Therefore, it is not possible to determine if this individual acquired the infection in the wild ecosystem or through indirect contact with peridomestic mosquitoes and infected dogs that accompany their owners to livestock and agricultural activities, a characteristic that is very common in dogs of people from rural municipalities from Yucatán state (Torres-Castro *et al*., 2022). This epidemiological aspect could influence the generation of the *D. immitis* transmission scenario in the populations of *N. narica* and other susceptible medium mammals (Moraes *et al*., 2017; Figuerêdo Duarte Moraes *et al*., 2022). Also, it has been argued that the human mobility and their domestic animals, mainly dogs, towards wild environments contributes to the exchange of zoonotic parasites such as *D. immitis* between different animal populations (wild and domestic) (Moraes *et al*., 2017).
Similarly, changes in land use, generated by activities such as urbanization, agriculture and livestock, cause the populations of *Nasua* sp. could coexist with wild canids that, in some endemic areas, have been described as competent hots of *D. immitis* (Simón *et al*., 2012; Penezić *et al*., 2014; Kotwa *et al*., 2019). In Yucatán, two species of wild canids are distributed: *Canis latrans* (colloquially known as “coyote”) and *Urocyon cinereoargenteus* (colloquially known as “gray fox”) (Sosa-Escalante *et al*., 2013). However, as far as we know, there are no records of *D. immitis* infection in these carnivore species, so more studies are necessary to establish their participation in the transmission cycle and spread of the parasite.
Another epidemiological factor that contributes to *D. immitis* transmission to atypical mammal hosts, such as *Nasua* sp., is the fragmentation of the natural habitat that allows adaptation to new environments and significant circulation of mosquitoes. In Yucatán, *Ae. taeniorhynchus* mosquitoes have been recognized as a competent vector of *D. immitis* (Manrique-Saide *et al*., 2008, 2010).
A hypothesis that should be included in future epidemiological studies is how the urbanization degree of the natural areas is modifying and influencing the transmission cycle of *D. immitis* through the generation of a higher rate of contact between populations of susceptible mammals, competent hots, and mosquito vectors (Worsley-Tonks *et al*., 2021). This wold help to know better the prevalence of *D. immitis* infection in coatis from the Yucatan Peninsula is substantial.

Conflict of Interest

Authors state no conflict of interest.

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