

Case Report

***Dirofilaria immitis* in Panama City: first molecular detection with public health implications**K. GONZÁLEZ^{1,*,#}, A. PÉREZ^{2,3}, C. AGUILAR⁴, A. VALDERRAMA⁵

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Summary

This report details a fatal case of canine dirofilariasis with concomitant *Ehrlichia canis* infection, emphasizing the role of molecular diagnostics and highlighting diagnostic and epidemiological gaps in Panama, particularly regarding stray dogs as key foci of infection. An 8-year-old mixed-breed dog presented with severe clinical signs, including adult nematode expectoration, lymphocytosis, eosinopenia, anemia, and thrombocytopenia. Rapid immunochromatographic tests confirmed co-infection with *Dirofilaria immitis* and *Ehrlichia canis*, with abundant microfilariae on blood smear. Echocardiography revealed severe right ventricular dilation, tricuspid regurgitation, and *D. immitis* adults in the heart and pulmonary artery. Necropsy confirmed severe right atrial rupture with hemopericardium and extensive parasitic burden, identified as *D. immitis* by molecular analysis (453 bp 16S rRNA product). This case underscores the advanced pathology of dirofilariasis and the aggravating role of co-infection, highlighting Panama's significant epidemiological challenges, including vector prevalence, stray-dog reservoirs, and limited access to therapy. These findings highlight the need for molecular diagnostics, comprehensive control strategies, and robust surveillance programs, especially for stray dog populations, to mitigate impacts on animal and public health.

Keywords: *Dirofilaria immitis*; dog; molecular analysis; Panama; public health; zoonosis

Introduction

Dirofilaria immitis, commonly known as heartworm, is an obligate parasitic nematode belonging to the family Onchocercidae (Canestri Trotti *et al.*, 1997). It's notable for its global distribution and the wide range of definitive hosts it infects, including domestic animals like canines (Anvari *et al.*, 2020), felines (Smith *et al.*, 2022), and various orders of wild mammals (Artiodactyla, Carnivora, Edentata, Lagomorpha, Perissodactyla, Primates, and Rodentia) (Dantas-Torres & Otranto, 2013). These parasites are transmitted by mosquito vectors, with at least 77 species identified as competent, including the genera *Culex*, *Aedes*, *Anopheles*, *Mansonia*, *Coquillettidia*, *Psorophora*, and *Culiseta* (Riahi *et al.*, 2021). This highlights the complexity and persistence of its transmission cycle.

In Latin America, vector-borne diseases are increasing and evolving as public health concerns. Dirofilariasis fits into this picture as an emerging zoonosis, worsened by a complicated interplay of the region's unique socio-environmental factors (Cardona-Ospina *et al.*, 2021). High population density, combined with the close cohabitation of domestic animals in both urban and rural settings, and a tropical climate that supports sustained proliferation of mosquito vectors for much of the year (Maggi & Krämer, 2019), creates an ideal epidemiological scenario for *D. immitis* persistence, transmission, and spread. This complex situation is further aggravated by large populations of stray and abandoned animals, particularly dogs. These animals not only act as key reservoirs but also facilitate parasite dispersal to new areas and significantly increase the risk of infection for other definitive hosts, including humans (Garde

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et al., 2013; Mota-Rojas *et al.*, 2021). Therefore, effective control of these zoonoses in the region is a crucial strategic priority to lessen their impact on public and animal health and to prevent new endemic areas from consolidating (Rodríguez-Morales *et al.*, 2021).

In Panama, dirofilariasis has garnered increasing attention, with initial reports in 2021 based on immunochromatographic tests (Candanedo Chacón & Candanedo, 2021). Subsequent research explored pulmonary and cardiac histopathological damage associated with *D. immitis* infection (Candanedo Chacón & Candanedo, 2023) and even confirmed the presence of the parasite in dogs with *Ehrlichia* spp. co-infections (Candanedo Chacón *et al.*, 2024) and, recently, a population genomics study of *D. immitis* that included sequences obtained from Panama (Power *et al.*, 2026). It is important to note that all these previous contributions focused solely on the western part of Panama. Despite Panama's critical epidemiological role as a "biological bridge" and biodiversity corridor, the lack of molecular diagnostic confirmation of *D. immitis* in pet and wild animal populations across various regions of the country has limited a precise characterization of the parasite and a comprehensive understanding of its true national distribution (Aththanayaka *et al.*, 2024). This deficiency not only hindered the implementation of an effective control strategy but also diminished Panama's ability to contribute robust data to regional and global epidemiological models. This report details a severe clinical case of an approximately 8-year-old rescued stray dog that presented with expectoration of an adult nematode. This led to a diagnosis of *D. immitis*, and post-mortem findings showed severe cardiac insufficiency. This case highlights the importance of comprehensive diagnosis and the severe pathological sequelae of advanced canine dirofilariasis.

Material and Methods

Case presentation: An approximately 8-year-old rescued stray female dog, of medium stature and 20 kg, presented with anorexia, lethargy, a temperature of 39.7°C, and palpable lymphadenopathy. The primary reason for consultation was the expectation of an adult nematode in sputum (Fig. 1A).

Blood examinations: A blood sample was collected by venipuncture from the *V. cephalica antebrachia* into tubes containing K₂EDTA and submitted for routine hematological examination using an automatic cell counter, the Mindray Auto Hematology Analyzer BC-5150 (Mindray Bio-Medical Electronics, Shenzhen, China). Whole blood was used in a rapid immunochromatographic test, Bioguard VLabs 4x® (Bioguard Corporation, New Taipei City, Taiwan), for antibodies against *Ehrlichia canis*, *Anaplasma* sp., and *Leishmania* sp., and for *Dirofilaria immitis* antigens. Finally, a thick blood smear was stained with the Diff-Quik procedure (Medion Diagnostics AG, Switzerland) for morphological identification of the microfilariae, using the dichotomous keys of Liotta *et al.* (2013).

Echocardiographic Evaluation: An echocardiographic examination was performed using a Samsung HS-40 ultrasound machine (Samsung Electronics, South Korea) with a 2 – 5 MHz convex transducer.

Necropsy: The necropsy was conducted following the guide established by McDonough and Southard (2017).

Molecular identification

To confirm the identity of the adult nematode, molecular characterization was performed. Genomic DNA was isolated using the MagMAX™ Viral/Pathogen II (MVP II) Nucleic Acid Isolation Kit (Applied Biosystems, Thermo Fisher Scientific, Waltham, MA, USA), following the manufacturer's instructions. Polymerase Chain Reaction (PCR) was executed to amplify a partial segment of the mitochondrial 16S rRNA gene, utilizing the *Dirofilaria immitis*-specific primers ROR 91 (5'-GCATCTTAGAACTTGGTC-CATCC-3') and ROR 92 (5'-CAAAGGCGTATTACCGCCAC-3') originally designed by Watts *et al.* (1999). The amplification profile followed the optimized thermal cycling conditions described by Vezzani *et al.* (2011). Assays were conducted using the HotStart

Table 1. Complete blood count (CBC) analysis of the affected canine patient. This table summarizes the whole blood hemogram data generated by the Mindray Auto Hematology Analyzer BC-5150. Key hematological parameters, including red blood cell indices, white blood cell differentials, and platelet counts, are provided with their respective units. Alterations from established canine reference intervals are highlighted, indicating, e.g., anemia, leukocytosis, and eosinophilia consistent with parasitic infection.

Parameter (Reference range)	Patient value
WBC (6 – 17)	16.76
Lym# (0.83 – 4.91)	11.65
Mon# (0.14 – 1.97)	1.29
Gran# (3.62 – 12.3)	3.82
Lym% (12 – 33)	69.5
Mon% (2 – 13)	7.7
Gran% (52 – 81)	22.8
RBC (5.1 – 8.5)	2.59
HGB (11 – 19)	8.9
HCT (33 – 56)	17.9
MCV (60 – 76)	69.1
MCH (20 – 27)	34.2
RDW (12.5 – 17.2)	25.8
PLT (200 – 490)	77
MPV (8 – 17.1)	12.6
PDW (12 – 17.5)	9.4
PCT% (0.09 – 0.58)	0.1
Eos% (0.5 – 10)	0.2
Eos# (0.04 – 1.62)	0.03

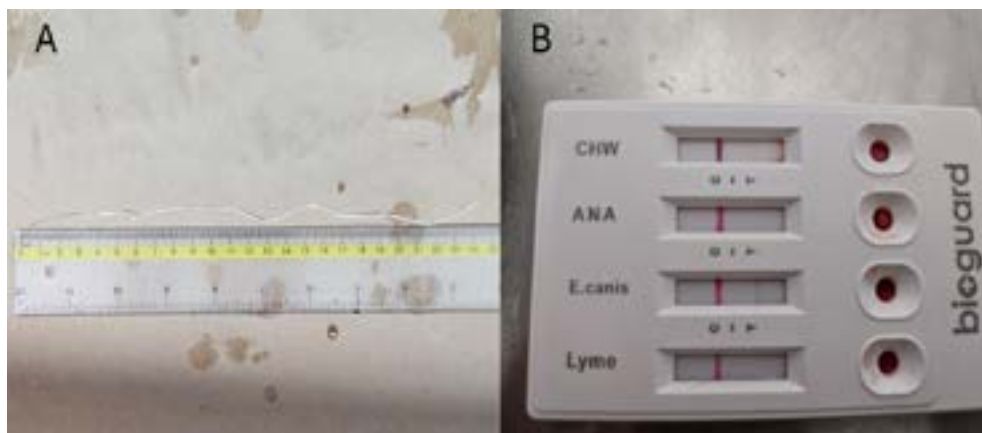


Fig.1. **A.** Adult *Dirofilaria immitis* nematodes expelled from a canine patient. An adult specimen of *Dirofilaria immitis*, approximately 24.5 centimeters in length, collected from the sputum expectorate by the patient. **B.** Bioguard Vlads 4Dx® rapid immunochromatographic test demonstrating co-infection. The positive line for *Ehrlichia canis* antibodies indicates previous exposure or active infection with *E. canis*. In contrast, the faint positive line for *Dirofilaria immitis* antigens (CHW) confirms the presence of the parasite.

Taq DNA Polymerase (BIORON GmbH, Römerberg, Germany). The reaction was initiated with a 10-minute denaturation step at 95°C to ensure complete enzyme activation. This was followed by 35 cycles of 95°C for 60 s, 55°C for 45 s, and 72°C for 60 s, with a final extension hold.

During the assembly pipeline, reads were assembled into a consensus contig using the CAP3 assembly algorithm integrated into UGENE software version 51.0 (Unipro LLC, Novosibirsk, Russia), followed by quality-based end-trimming to remove low-confidence noise from both ends. This bioinformatic procedure yielded a high-fidelity 249-bp consensus fragment, which was subsequently used for definitive taxonomic identification via the Basic Local Alignment Search Tool (BLASTn).

Phylogenetic analysis

The sample sequence was assembled, visually checked, and trimmed to the partial 16S rRNA region using Geneious Prime 2026.0.01 (Kearse *et al.*, 2012). The resulting sequence was aligned with previously published 16S rRNA data from *Dirofilaria* specimens, as well as other closely related species, and with *Ascaris* sp. (MN914077) as the outgroup, using MAFFT's (Kato *et al.*, 2019) L-INS-i translational alignment plugin of Geneious. The phylogenetic tree was estimated using the maximum-likelihood criterion in IQ-TREE (Minh *et al.*, 2020). The best-fitting nucleotide-evolution model was selected using the corrected Akaike information criterion in IQ-TREE, and nodal supports were estimated by running 1,000 standard nonparametric bootstrap replicates.



Fig. 2. *Dirofilaria immitis* microfilaria in a peripheral blood smear (Hematoxylin-Eosin stain). This photomicrograph, taken at 1000X oil-immersion, shows a single *Dirofilaria immitis* microfilaria in a peripheral blood smear. The microfilaria exhibits a fusiform body, a distinct diffuse nuclear distribution, and a notable tapered caudal extremity, consistent with the morphology of *D. immitis*.

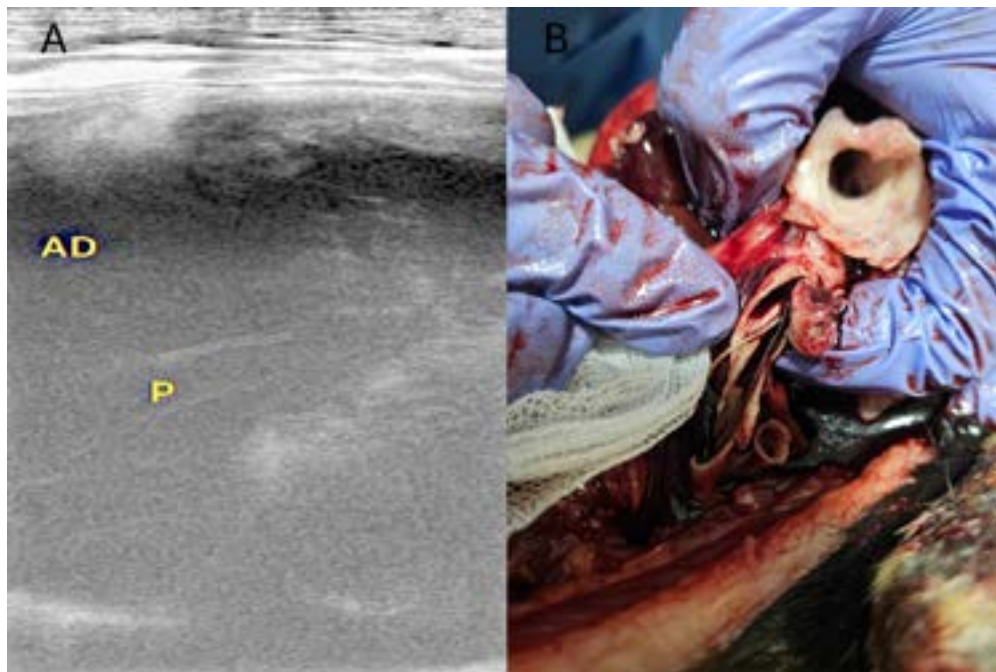


Fig. 3. **A.** Echocardiographic visualization of adult *Dirofilaria immitis* in the right atrium. This ultrasound image captures the characteristic hyperechoic, parallel lines of adult *D. immitis* (P) within the lumen of the patient's right atrium (AD). **B.** Macroscopic view of adult *Dirofilaria immitis* nematodes within the pulmonary artery.

Ethical Approval and/or Informed Consent

The authors declare that all examinations performed were within the scope of a veterinary examination.

Results

A complete blood count (CBC) revealed lymphocytosis and eosinopenia in the white cell line, anemia in the red cell line, and thrombocytopenia (Table 1). These hematological findings suggested a chronic inflammatory response, bone marrow suppression, and potential platelet consumption, all of which are common in co-infections with other pathogens.

The rapid immunochromatographic test was positive for antibodies against *Ehrlichia canis* and for *Dirofilaria immitis* antigens, thereby confirming co-infection (Fig. 1B). Subsequently, the thick blood smear revealed abundant peripheral blood microfilariae (+++), corroborating active *D. immitis* infection (Fig. 2).

On ultrasonography, mobile, hyperechoic, linear structures consistent with adult *D. immitis* parasites were visualized within the right atrium and pulmonary artery (Fig. 3A). Additionally, severe right ventricular dilation was observed, along with moderate tricuspid regurgitation and findings consistent with severe pulmonary hypertension. These findings indicated significant hemodynamic compromise and severe cardiac dysfunction secondary to the parasitic burden.

Necropsy revealed the extensive and severe nature of the disease.

A severe dilation of the right atrium was found, with rupture of the atrial wall and presumed extravasation of blood into the pericardial cavity (hemopericardium). This finding is consistent with atrial rupture secondary to chronic pressure overload, a mortal complication of advanced dirofilariasis. Numerous adult nematodes were observed within the right cardiac chambers (atrium and ventricle) as well as in the pulmonary artery (Fig. 3B). Obstruction of blood flow and hemodynamic overload from this massive parasitic infestation were the primary factors contributing to the dilation and eventual rupture of the right atrium.

At the molecular level, a 453-base-pair PCR product was successfully obtained, and the sequence was deposited in GenBank under accession number PX563298.

Maximum-likelihood phylogenetic analysis confirmed the species identification as *D. immitis*, placing the specimen at the base of a well-defined clade composed exclusively of *D. immitis* representatives (Fig. 4).

Discussion

The clinical signs observed in this case, including lethargy, anorexia, and the expectoration of an adult nematode, are highly consistent with the course of canine filariasis caused by *D. immitis* (Atwell, 2018). The severity of the condition, culminating in severe cardiac insufficiency and fatal atrial rupture, is compatible with Stage III dirofilariasis, which is associated with a reserved to unfavorable prognosis (Polizopoulou *et al.*, 2000). In addition to

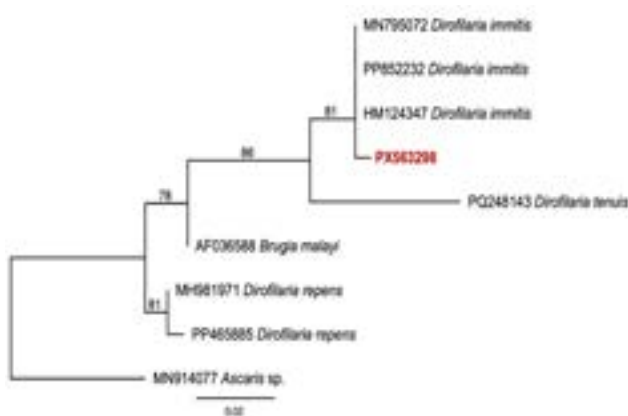


Fig. 4. Phylogenetic tree inferred by the Maximum Likelihood method and the HKY+F+I evolutionary model based on an alignment of 249 bp of the 16S rRNA gene. Sequence from the present study is highlighted in bold red. *Ascaris* sp. was used as an outgroup, and node numbers indicate bootstrap values.

this clinical complexity, a co-infection with *E. canis* was identified, an association previously reported in Panama's Chiriquí province (Candanedo Chacón *et al.*, 2024). The presence of multiple vector-borne pathogens can significantly worsen the patient's condition, adversely affecting clinical presentation, disease progression, and hematological findings through a potential synergistic effect (Candanedo Chacón *et al.*, 2024; Dantas-Torres & Otranto, 2013). The patient's history of street rescue is a crucial factor and a significant limitation in this case. The absence of prior medical history and prophylaxis, coupled with constant exposure to an uncontrolled environment, likely contributed to the acquisition of these infections. This situation underscores the vulnerability of homeless animals, which not only suffer the consequences of neglected diseases but also act as reservoirs and sources of infection for other animals and, potentially, humans, within a One Health perspective (Nemetov *et al.*, 2025). The lack of long-term follow-up and the impossibility of specific treatment for dirofilariasis due to the patient's acute deterioration further highlight the therapeutic challenges in the region. Critically, a significant limitation in Panama is the absence of government-approved, specific adulticidal medications for canine dirofilariasis on the local market. This severely hinders the complete and effective therapeutic management of the disease in the country, especially in areas with socioeconomic difficulties, where the owners cannot afford the government-approved prophylactic compounds on the market with proven efficacy. This barrier not only impacted the prognosis of this particular animal but also represents a constant and significant challenge for animal health in the region, underscoring the difficulties in accessing essential treatments for such a dangerous disease.

In Panama, mosquito vectors pose an epidemiological risk factor for multiple diseases. While the vector competence of local species for *D. immitis* has not been demonstrated, there is evidence that some species can serve as carriers of filarial parasites, including *Dirofilaria repens*, in Darien province, Panama (Rivera *et al.*,

2025). Genera such as *Culex* and *Aedes* are known as confirmed dirofilariasis vectors in other regions (Ledesma & Harrington, 2011). In Panama's urban environment, highly anthropophilic species such as *Culex quinquefasciatus* and *Aedes aegypti* are widely circulating, alongside other *Culex* and *Aedes* species (Whiteman *et al.*, 2019; Lopez-Solis *et al.*, 2023). Although their primary role in *D. immitis* transmission to humans in the region is not fully elucidated, the ability of these mosquito species to colonize new ecological niches and invade human dwellings (Loaiza *et al.*, 2017) poses a potential risk not only for zoonotic transmission but also for maintaining the disease cycle within the country.

Conclusion

This case underscores the relevance of targeted molecular tools for the definitive diagnosis of canine dirofilariasis and for providing reliable epidemiological data. The selection of the mitochondrial 16S rRNA gene as a molecular marker offered distinct analytical advantages over other conventional loci; unlike the highly conserved nuclear 18S rRNA gene or the *cox1* gene, which is frequently hindered by the co-amplification of nuclear pseudogenes (Numts) in nematoda, the 16S rRNA locus provided clean, high-fidelity reads with ideal species-level resolution. Confirming the presence of *D. immitis* by capturing this specific hypervariable region significantly strengthens the region's diagnostic precision and collaborative epidemiological surveillance capacity.

Collaboration between clinics and reference centers, such as the Gorgas Memorial Institute (ICGES), is crucial for supporting the surveillance and control of canine dirofilariasis and other zoonotic diseases, as well as addressing their public health implications in Panama. More studies are needed to understand the distribution of this parasite and the extent of the threat it poses to public health in Panama.

This work represents the first molecular identification of *Dirofilaria immitis* in Panama City.

Conflict of Interest

The authors state there is no conflict of interest.

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