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Feline heartworm disease in endemic settings: an integrated diagnostic approach

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ABSTRACT

Feline heartworm disease, caused by *Dirofilaria immitis,* is an emerging concern in endemic regions, although its epidemiology remains poorly defined due to diagnostic limitations. In Italy, available data on feline *D. immitis* infection are limited and largely based on serological investigations. The aim of the present study was to provide an epidemiological assessment of infection in cats across Sardinia, a region where canine dirofilariosis is endemic. A total of 141 cats were evaluated using a multimodal diagnostic approach including echocardiography, antigen detection, and the modified Knott's test. In addition, antibody detection by in-house ELISA and PCR targeting the 16S rRNA gene of endosymbiont *Wolbachia pipientis* were performed to deeply investigate the infection features. All cats underwent physical examination and clinical findings were recorded. Evidence of *D. immitis* infection was detected in 2.8% of cats by echocardiography, corresponding to the overall prevalence observed in the study, and in 2.1% by antigen detection, while microfilariae of *D. immitis* were detected in 0.7% of cats. Antibodies to *Dirofilaria* spp. were found in 14.9% of animals, indicating broader exposure. *Wolbachia* DNA was identified in 1.4% of samples. A significant association was observed between *D. immitis* positivity and the presence of compatible clinical signs such as coughing, dyspnea, and vomiting ($P = 0.016$). These findings confirm the presence of feline heartworm infection in Sardinia, highlighting the diagnostic complexity of the disease and the importance of prevention in endemic areas, given the severity of this parasitosis in cats and the current lack of an effective therapy.

1. Introduction

Dirofilaria immitis, together with *Dirofilaria repens,* is a zoonotic, mosquito-borne filarial parasite that primarily infects dogs, but also cats, wild carnivores, and humans (Simón et al., 2022; Perles et al., 2024), demonstrating poor vertebrate host specificity (Barriga, 1982). In companion animals, *D. immitis* is responsible for canine and feline heartworm disease (HWD), whereas *D. repens* causes canine and feline subcutaneous dirofilariosis (SCD; Simón et al., 2012). Feline infection with *D. immitis* is characterized by transient or absent microfilaremia (i.e., occult infection) and by aberrant localization of adult worms (e.g., to body cavities, the central nervous system, or the femoral artery; McCall et al., 2008; Simón et al., 2012; Favole et al., 2013; Oldach et al., 2018). Early death (3–4 months post-infection) of juvenile heartworms usually occurs soon after they arrive in the pulmonary arteries; otherwise, they survive about 2–4 years, followed by spontaneous self-cure of the infection by the host in most cases (Venco et al., 2008). Early or end-stage parasite death can be associated with acute severe lesions

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(severe pulmonary thromboembolism and eosinophilic inflammatory response in lungs) causing the so-called heartworm-associated respiratory disease (HARD), characterized by acute onset of dyspnoea and interstitial pattern on lung radiography, which is often fatal (Venco et al., 2015; Pennisi et al., 2020).

Feline infections have been reported in the same geographical areas where canine HWD is endemic, with estimated prevalence in cats corresponding to approximately 9–18% of those observed in dogs (Venco et al., 2011). However, several studies investigating anti-*D. immitis* antibodies in cats have demonstrated substantially higher exposure rates than those suggested by prevalence (Venco et al., 2011, 2015; Diakou et al., 2019; Pennisi et al., 2020), emphasizing the importance of local epidemiological dynamics when assessing the risk of feline infection. Insular settings such as Sardinia (Italy) represent geographically defined ecological systems that facilitate the characterization of parasite circulation (Foufopoulos et al., 2016). A recent large-scale epidemiological study conducted in dogs across the island reported a prevalence of 9.9% for *D. immitis* and 5.5% for *D. repens* (Nonnis et al., 2025), confirming the endemic circulation of both filarial species. Hyperendemic foci have been documented on the island, with prevalence peaks of 17.1% for *D. immitis* in Oristano province (Scala et al., 2004) and 27.3% and 18.5% for *D. immitis* and *D. repens*, respectively, in San Pietro Island (Traversa et al., 2019). Together, these findings indicate sustained parasite circulation and suggest that cats sharing the same environment with dogs may also be exposed to infection, as supported by nationwide questionnaire-based studies reporting feline involvement in dirofilariosis in Sardinia (Genchi et al., 2019; Genchi et al., 2023). This study aimed to provide the first epidemiological assessment of *D. immitis* infection in cats across Sardinia, contributing essential data for a region known to be endemic for canine dirofilariosis. To improve the detection of infection in a species in which HWD frequently occurs in an occult form, a multimodal diagnostic approach including echocardiography, antigen detection and Knott's test was implemented. Additionally, antibody testing and biomolecular detection of *Wolbachia pipientis* were performed to provide a more comprehensive characterization of the infection.

2. Materials and methods

2.1. Study design and animals

According to the Banca Dati Nazionale Animali da Compagnia (Banca Dati Nazionale Animali da Compagnia Regione Autonoma della Sardegna, 2025), the number of owned registered cats in Sardinia amounts to 13,476; however, the stray and colony cat population is presumed to be substantially larger. Indeed, approximately 500 feline colonies, including unregistered colonies, are estimated to be present across the island. The Sardinian regional authorities annually support sterilization programs for stray and colony cats, with 765 surgical procedures reported in the most recent available data from 2022 (Regione Autonoma della Sardegna, 2025). Based on these data, the sample size was calculated considering both the number of owned registered cats and the annual number of sterilization surgeries performed in stray or colony cats in Sardinia.

An expected prevalence of 10% was conservatively assumed to maximize variability. The calculation was performed considering a 95% confidence interval and a 5% margin of error, resulting in an estimated sample size of 137 subjects (http://www.raosoft.com/samplesize.html).

Animals were enrolled among cats presented to the Veterinary Teaching Hospital (Department of Veterinary Medicine, University of Sassari) and/or to other contracted private veterinary clinics for routine veterinary visits, ovariohysterectomy or health issues. Only cats fulfilling the predefined inclusion criteria were enrolled. Eligible animals were required to have regular outdoor access, to be at least 1 year old and have experienced one mosquito transmission season, and to have received no parasiticides active against *Dirofilaria* spp., nor ectoparasiticides or repellents, during the 6 months preceding sampling, to avoid interference with their infection status.

Written informed consent for the enrolment of animals was obtained from owners, shelter managers, or authorized representatives of animal welfare associations. All procedures were conducted in compliance with animal welfare regulations, and the study protocol was reviewed and approved by the Ethics Committees of the University of Sassari, Italy (protocol no. 54189, 27 May 2025).

2.2. Clinical and diagnostic procedures

2.2.1. Physical examinations and blood sampling

At enrolment, an individual record was compiled for each cat, including signalment (age and sex) and ownership status. Cats were classified by age as young (1–6 years), mature (6 years and 1 month to 10 years), and senior (>10 years). Information on known or previously diagnosed diseases was also collected. All enrolled cats underwent a physical examination, during which clinical findings including general health status, body condition, and any abnormalities or signs suggestive of *D. immitis* infection or other diseases were recorded. During the clinical procedures, blood samples were collected in tubes with and without anticoagulant (K3EDTA) to obtain serum for antigen and antibody detection and whole blood for the modified Knott's test and molecular analyses. Antigen testing and the modified Knott's test were performed within 48 h of sampling at the Laboratory of Parasitology and Parasitic Diseases of the Department of Veterinary Medicine of the University of Sassari. Aliquots of serum and whole blood intended for antibody detection and PCR (polymerase chain reaction) were stored at −20 °C and subsequently shipped to the Department of Public Health and Infectious Diseases of Sapienza University of Rome for further analyses.

2.2.2. Echocardiographic examination

All cats underwent a standard echocardiographic examination (Thomas et al., 1993) performed by experienced operators using different ultrasound systems, including Vivid T8 Ultra and Vivid iQ (GE Healthcare, Chicago, IL, USA), equipped with 12S (5–11 MHz) and 6S (2.7–8 MHz) cardiac probes, a Sequoia ultrasound system (Siemens) equipped with a 10 V4 transducer (TRANSDUCER, 10 V4, CP), and a Mindray M11 ultrasound system equipped with a P8–2 probe. During echocardiographic examination attention was paid to the 2D evaluation of the right heart, main pulmonary artery, and the explorable portions of its two main branches in order to identify any parasites (DeFrancesco et al., 2001).

2.2.3. Antigen detection

Serum samples were tested for the detection of *D. immitis* female antigens using a commercial ELISA (enzyme-linked immunosorbent assay) kit (SNAP® Feline Triple, IDEXX Laboratories, Westbrook, ME, USA) according to the manufacturer's instructions. Briefly, three drops of serum were mixed with four drops of conjugate and processed using the SNAP® device; results were read and recorded after 8 min. The SNAP® Feline Triple test has a reported sensitivity (se) of 90.2% and specificity of 100% and, in addition to *D. immitis*, allows the detection of antibodies against feline immunodeficiency virus (FIV) and antigen of feline leukemia virus (FeLV).

2.2.4. Knott's test on blood sample

Uncoagulated whole blood samples were analyzed using the modified Knott's test as previously described (Knott, 1939). Briefly, 1 ml of whole blood was mixed with 9 ml of distilled water in a conical centrifuge tube and centrifuged for 5 min at 1500 rpm. The supernatant was discarded and the sediment stained with one drop of 1% methylene blue. The stained sediment was then transferred onto a glass slide and the whole sediment was examined under light microscopy at 5× magnification. Detected microfilariae (mfs) were examined at 20× and

40× magnification and measured with a digital image processing system (LC Micro Image Acquisition Software v.5.2, Olympus Corporation, Hachioji, Tokyo, Japan), identified to the species level based on morphometric criteria (Magnis et al., 2013), and subsequently counted, with parasite burden expressed as mfs per milliliter of blood (mfs/mL).

2.2.5. Antibody detection

Serum samples were tested to assess exposure to *Dirofilaria* spp. using an in-house ELISA based on somatic antigens of adult *D. immitis*, with a final concentration of 0.4 μg/μl (Perles et al., 2025). Sera were tested in solid-phase ELISA at a dilution 1:200 and goat anti-cat-IgG conjugated to horseradish peroxidase (Sigma-Aldrich, St. Louis, MO 63118, USA) was used as a secondary antibody at 1:20,000 dilution. The optical density (OD) was measured at 450 nm (Easy-Reader, Bio-Rad, Hercules, CA, USA). The cut-off point (OD = 1.15) was established by calculating the mean value +3 S.D. of 100 control serum samples from seronegative and PCR-negative cats living in a non-endemic region. The in-house ELISA employed had 89% Se and 98% Sp (Perles et al., 2025).

2.2.6. Molecular testing

Genomic DNA was extracted from whole blood samples after thawing at room temperature using the QIAamp DNA Blood Mini Kit (Qiagen, Milan, Italy), following the manufacturer's protocol and subjected to further screening for the endosymbiont *Wolbachia* by qPCR targeting the 16S rRNA gene, as described by Willems et al. (1999). All PCR products were visualized on a 2% agarose gel stained with Gel Red® nucleic acid gel stain (VWR International PBI, Milan, Italy) and documented using a Gel Logic 100 imaging system (Kodak, New York, NY, USA).

2.3. Statistical analysis

The cumulative prevalence of *D. immitis* infection was calculated by considering cats testing positive on at least one of the direct diagnostic assays employed. Differences in cumulative positivity according to sex, age, and housing conditions, were analyzed to identify potential risk factors, with statistical significance set at $P < 0.05$. Statistical analyses were also performed to evaluate possible associations between *D. immitis* positivity (yes/no) and clinical signs. Additional analyses investigated the potential relationship between *D. immitis* and FIV/FeLV status as determined by the SNAP® Feline Triple test (IDEXX Laboratories, Westbrook, ME, USA).

3. Results

The study population included 141 cats (62 females and 79 males), of which 89 were enrolled at the Veterinary Teaching Hospital and 52 at private clinics. Based on age classification, 77 cats were categorized as young, 24 as mature, and 40 as senior. Sixty-four cats were privately owned, whereas 77 originated from shelters or feline colonies. Among the enrolled animals, 7.1% (10/141) presented clinical signs potentially consistent with cardiopulmonary dirofilariosis, including cough, dyspnea, and vomiting. Of these clinically affected cats, 20% (2/10) tested positive for *D. immitis*.

The main clinical and diagnostic findings for the cats positive for *D. immitis* are summarized in Table 1.

3.1. Echocardiographic examination for the detection of D. immitis

On echocardiographic examination, *D. immitis* infection was identified in 2.8% (4/141) of the cats. In all positive cases, paired hyperechoic linear structures compatible with parasite segments were observed within the lumen of the main pulmonary artery and right pulmonary artery branch (Fig. 1). Cardiac chambers were normal in size, and no valvular insufficiencies or other relevant echocardiographic abnormalities were detected.

3.2. Antigen detection

Antigen testing identified *D. immitis* infection in 2.1% (3/141) of the cats. Using the same assay, 12.8% (18/141) and 3.5% (5/141) of animals tested positive for FIV antibodies and FeLV antigen, respectively.

3.3. Knott's test on blood sample

Overall, 2.1% (3/141) of cats tested positive on the modified Knott's test. Microfilariae of *D. immitis* were identified in one case only (0.7%; Fig. 2), in a co-infection with *D. repens*, that were detected in all positive samples (2.1%).

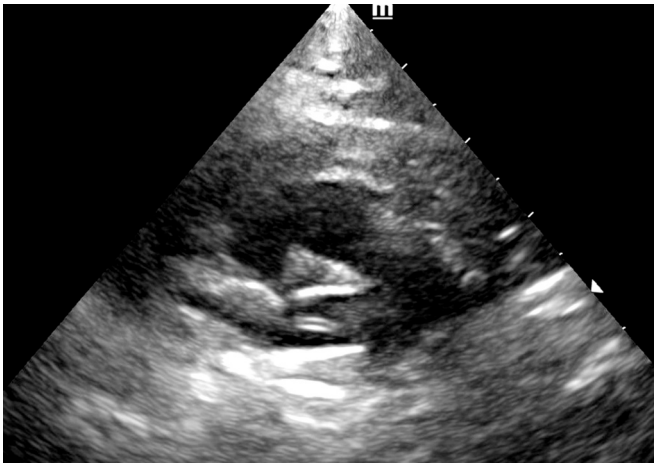

Fig. 1. Right parasternal short-axis view at the level of the heart base optimized for pulmonary artery visualization. Two hyperechoic parallel linear structures are visible within the right pulmonary artery branch, appearing as echogenic bands floating within the vessel lumen, consistent with worm segments.

Table 1
Clinical characteristics and diagnostic findings in the four cats positive for *Dirofilaria immitis*.

ID	Sex	Age (months)	Ownership status	Clinical signs	Echocardiography	Antigen of *D. immitis*	Felv/Fiv status	Knott's Test	IgG anti-*Dirofilaria* spp. (titer)	PCR *Wolbachia* spp.
1	M	48	Owned	0	1	1	Fiv+	*D. immitis* (2 mfs/ml). *D. repens* (783 mfs/ml)	3.45	1
2	M	30	Owned	Cough, dyspnea	1	1	0	0	3.8	0
3*	F	108	Owned	0	1	1	0	0	3.6	0
4	M	84	Owned	Asthma, vomiting	1	0	0	0	0	0

* Cat died several months after diagnosis; causality with *D. immitis* infection could not be confirmed.

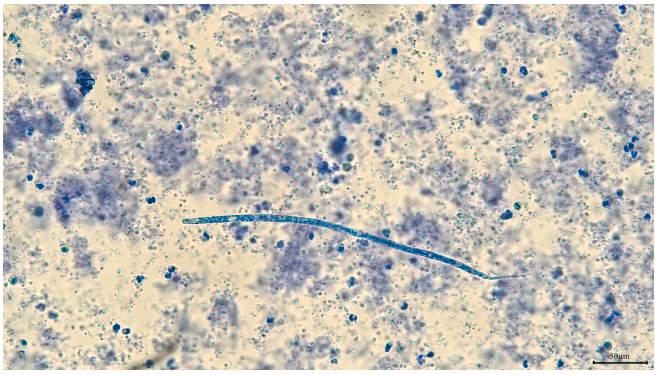

Fig. 2. Light microscopy image (20× magnification) showing a microfilaria of *Dirofilaria immitis*.

3.4. Antibody detection

Anti-*Dirofilaria* spp. antibodies were detected in 14.9% (21/141) of the tested cats using the in-house ELISA. Positive sera showed optical density values ranging from 1.44 to 3.83, all above the predefined cut-off (OD = 1.15).

3.5. Molecular testing

PCR analysis targeting the 16S rRNA gene of *Wolbachia* detected *Wolbachia* DNA in 1.4% (2/141) of the cats. Amplified products were successfully visualized by agarose gel electrophoresis.

3.6. Statistical analysis

Infection was not significantly associated with sex ($\chi^2 = 0.07$, $P = 0.791$), with prevalences of 3.8% (3/79) in males and 1.6% (1/62) in females. Similarly, age was not significantly associated with infection (χ^2-test for trend, $\chi^2 = 0.303$, $P = 0.582$). Although a higher prevalence was observed in mature cats (8.3%, 2/24; OR = 3.409) compared to young cats (2.6%, 2/77; OR = 1.000), no positive cases were detected in senior cats (0/40; OR = 0). Ownership status was also not significantly associated with infection ($\chi^2 = 2.945$, $P = 0.086$), although all positive animals were owned cats (6.3%, 4/64), while no cases were identified among shelter or colony cats (0/77).

In contrast, a significant association was observed between *D. immitis* positivity and the presence of clinical signs compatible with cardiopulmonary dirofilariosis such as dyspnea, coughing, and vomiting ($\chi^2 = 5.777$, $P = 0.016$). Symptomatic cats showed a markedly higher prevalence (20.0%, 2/10) compared to asymptomatic animals (1.5%, 2/131).

No significant association was observed between *D. immitis* positivity and FIV or FeLV status ($\chi^2 = 0$, $P = 1.000$).

4. Discussion

The present study provides the first epidemiological assessment of feline heartworm infection in Sardinia using a multimodal diagnostic approach, confirming the presence of *D. immitis* in cats living in a region already recognized as endemic for canine dirofilariosis and supporting the ongoing circulation of this zoonotic parasite on the island. Evidence of infection was identified by echocardiography in 2.8% of cats and by antigen testing in 2.1%, while mfs of *D. immitis* were detected in 0.7% of cats. All cats positive by antigen detection and modified Knott's test were also positive at echocardiographic examination; therefore, the overall prevalence corresponded to the prevalence detected by echocardiography. Antibodies against *Dirofilaria* spp. were detected in 14.9% of animals, indicating broader exposure within the studied population. Additionally, *Wolbachia* DNA was identified in 1.4% of samples.

The heterogeneous diagnostic patterns observed among infected cats underscore the intrinsic complexity of feline heartworm disease. Despite growing awareness, antemortem diagnosis in this species remains challenging (DeFrancesco et al., 2001). Discordant results among imaging, antigen and antibody testing, mfs detection, and PCR for *W. pipientis* reflect the biological characteristics of infection in cats, typically characterized by low worm burdens and transient microfilaremia (Simón et al., 2012). Consequently, current guidelines recommend a multistep diagnostic strategy to overcome the limitations of each single diagnostic technique, in which thoracic radiography and antibody testing help raise suspicion of infection, whereas echocardiography combined with antigen testing and microfilariae detection is used to confirm or exclude the presence of adult parasites (European Society of Dirofilariosis and Angiostrongylosis (ESDA), 2017; Pennisi et al., 2020).

The overall prevalence detected herein (2.8%) aligns with the expectation that feline infection in an endemic setting falls within the theoretically expected limits of 9–18% of canine prevalence (Venco et al., 2011), considering that canine prevalence in Sardinia has been reported at 9.9% using the modified Knott's test and 12.2% by antigen testing (Nonnis et al., 2025). Dogs remain the primary reservoir hosts for these parasites (Simón et al., 2012), whereas cats play a comparatively minor role in transmission, being less frequently infected and typically harboring lower parasite burdens (Venco et al., 2011). Nevertheless, evaluation of feline infection remains epidemiologically relevant in endemic regions where chemoprophylaxis is systematically implemented in dogs, as alternative hosts such as cats may contribute to maintaining endemicity (Ciuca et al., 2020; Panarese et al., 2021).

In Italy, studies investigating feline *D. immitis* infection remain relatively limited and have been based predominantly on serological surveys, either alone or in combination with selected diagnostic methods such as the modified Knott test, antigen detection, or PCR (Kramer and Genchi, 2002; Magi et al., 2002; Traversa et al., 2010; Giangaspero et al., 2013; Brianti et al., 2022; Grillini et al., 2022; Genchi et al., 2024; Carbonara et al., 2025). In Sardinia, where the first feline case was reported in 2009 (Scala et al., 2009), available epidemiological data have likewise been derived mainly from serological investigations. A study conducted in the province of Oristano in 2010 detected anti-*D. immitis* antibodies in 14% of examined cats (Scala et al., 2010), whereas more recently Genchi et al. (2024) reported a seroprevalence of 20.8% in a multicenter survey including cats from Sardinia, representing the highest seroprevalence recorded among Italian regions.

Ultrasonography is a well-recognized tool in the diagnosis of parasitic diseases in veterinary medicine (Corda et al., 2022). In the present study, echocardiography identified *D. immitis* nematodes in 2.8% of examined cats, accounting for all the confirmed infections detected among the diagnostic methods applied. Echocardiography allowed direct visualization of paired hyperechoic linear structures within the right heart and the pulmonary arteries, providing definitive antemortem evidence of *D. immitis* presence (Litster and Atwell, 2008; Lee and Atkins, 2010). However, its diagnostic performance remains strongly operator-dependent, with reported sensitivities ranging from 40% to 100% (DeFrancesco et al., 2001). Furthermore, the absence of detectable worms does not exclude infection, and false-positive interpretations may occur due to imaging artefacts or misidentification of normal cardiac structures (Atkins et al., 2008). Despite these limitations, echocardiography proved to be the most successful tool for confirming *D. immitis* infection in this study.

Antigen testing identified infection in a slightly lower proportion of cats compared to echocardiography. This discrepancy likely reflects the biological characteristics of feline infection, which is frequently associated with low worm burdens and male worm infections (Berdoulay et al., 2004). Antigen assays detect circulating mature female antigens and therefore indicate active infection; however, negative results may occur in cases involving only male worms, a single female, immature

parasites, or aged adult worms (McCall et al., 1995; Piché et al., 1998; Venco et al., 2011).

Microfilariae of *D. immitis* were detected in only 0.7% of cats, confirming that circulating mfs are rare in feline heartworm infection. Fewer than 20% of infected cats develop detectable microfilaremia, and when present, it is typically transient, lasting only 1–2 months after patency (7–8 months post-infection; McCall et al., 2008; Simón et al., 2012).

Serological analysis revealed a higher exposure rate compared with direct diagnostic methods. Antibody positivity may reflect early infection, repeated exposure to infective larvae, or past infection rather than the presence of mature parasites (Atkins et al., 2000; Villanueva-Saz et al., 2021). Consistently, only 10–20% of seropositive cats are estimated to harbor adult worms within the right heart and pulmonary arteries (Miller et al., 2000), explaining why the proportion of echocardiography-positive cats observed in this study aligns with expectations based on the detected seroprevalence. Antibody testing presents inherent limitations. Although it reflects exposure, it does not confirm active infection, and antibody titers may decrease after parasite maturation, leading to seronegative results in infected cats (American Heartworm Society, 2024; Venco et al., 2011). This finding was observed in the present study, where one echocardiography-positive cat tested seronegative. Such discordance may be attributable to testing prior to seroconversion (approximately 3–6 months post-infection), declining antibody levels during chronic infection, or host immunosuppression (Dillon, 1998). Furthermore, the serological assay employed, based on somatic antigens, does not reliably differentiate between *D. immitis* and *D. repens*, limiting their diagnostic specificity when used in isolation (Perles et al., 2025).

PCR-based detection of *W. pipientis* DNA was positive in 1.4% of cases, but only one cat tested *D. immitis* positive while the second was only positive for *D. repens* mfs. The detection of *Wolbachia* DNA in microfilaremic cats agrees with a previous study in dogs stating that the molecular detection of this endosymbiont is associated with circulating mfs (Louzada-Flores et al., 2022).

Statistical analysis demonstrated a significant association between *D. immitis* positivity and the presence of clinical signs consistent with feline HWD, with symptomatic cats showing a higher prevalence than asymptomatic animals. All infected cats were privately owned; although ownership status was not statistically significant, this pattern may reflect differential access to veterinary care rather than true epidemiological risk, as owned cats are more likely to undergo clinical evaluation, particularly when presenting with respiratory or systemic signs, thereby increasing the probability of infection detection (Robertson-Plouch et al., 2000).

Overall, the findings of the present study further highlight the diagnostic complexity of feline heartworm disease. Consistent with current guidelines (European Society of Dirofilariosis and Angiostrongylosis (ESDA), 2017; Pennisi et al., 2020), our results support the need for a multimodal diagnostic approach combining serological testing and imaging techniques. Among the methods applied, echocardiography and antigen testing showed the highest diagnostic yield. In our study, echocardiography alone would have been sufficient to identify all confirmed infections; however, its routine use in clinical practice may be limited by the cost of the procedure and the need for experienced operators. Therefore, a practical diagnostic strategy may consist of initial antigen testing, followed by echocardiographic evaluation when clinical suspicion persists despite negative antigen results. In contrast, the modified Knott's test showed limited diagnostic value, as previous highlighted (Venco et al., 2015; Pennisi et al., 2020).

Management of feline heartworm disease remains challenging. Current guidelines indicate that no adulticidal therapy has been shown to improve survival in cats harboring adult *D. immitis*, and such treatments may be associated with severe complications (McCall et al., 2008). Consequently, management is generally conservative and based on symptomatic therapy and periodic monitoring (Lee and Atkins, 2010; Venco et al., 2015; Pennisi et al., 2020). In the present study, one of the infected cats died several months after diagnosis; although a causal relationship with heartworm infection could not be established, this observation reflects the potentially severe and unpredictable nature of the disease even in animals with a low parasite burden (Litster and Atwell, 2008). In this context, prevention remains the cornerstone of disease control, particularly in endemic areas such as Sardinia where *D. immitis* actively circulates. Regular administration of macrocyclic lactones targeting infective larvae is therefore recommended, including for indoor cats (European Society of Dirofilariosis and Angiostrongylosis (ESDA), 2017; American Heartworm Society, 2024).

Although the primary objective of this study was the assessment of *D. immitis* infection, the detection of *D. repens* mfs in 2.1% of examined cats further confirms the circulation of filarial species within the feline population of Sardinia. Epidemiological data on feline *D. repens* infection remain limited (Villanueva-Saz et al., 2024), and clinical presentation ranges from asymptomatic to dermatological manifestations such as nodules, pruritus, and erythema (Pennisi et al., 2020). In cats, this subcutaneous filarial species has been reported mainly through cytological evaluation of cutaneous lesions or as an incidental finding during elective surgical procedures (Ciuca et al., 2020; Villanueva-Saz et al., 2024). Given the zoonotic relevance of *D. repens*, its detection in cats even at low prevalence supports the inclusion of feline hosts in the broader epidemiological framework of filarial infections in endemic areas (Simón et al., 2012).

5. Conclusion

Feline heartworm disease remains a diagnostic challenging condition due to the low parasite burden and the frequent discordance among available tests. As also highlighted by the present study, reliable diagnosis often requires a multimodal approach combining antigen testing and imaging techniques. In the absence of a safe and effective adulticidal therapy, clinical management is mainly conservative, making prevention the cornerstone of disease control. From an epidemiological perspective, our results confirm the circulation of *D. immitis* in the feline population of Sardinia, with evidence of infection detected by echocardiography in 2.8% of cats, while antibodies against *Dirofilaria* spp. were detected in 14.9% of animals, indicating broader exposure within the studied population. Preventive administration of macrocyclic lactones should therefore be recommended for cats, particularly in areas endemic for canine dirofilariosis such as Sardinia, but also in other regions where exposure to infected mosquitoes may occur, including in indoor animals.

CRediT authorship contribution statement

Francesca Nonnis: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Andrea Corda:** Writing – review & editing, Supervision, Methodology, Investigation. **Pamela Zeinoun:** Writing – review & editing. **Lia Cavallo:** Writing – review & editing. **Francesca Corda:** Writing – review & editing, Investigation. **Francesca Cubeddu:** Writing – review & editing, Investigation. **Stefano Rocca:** Writing – review & editing. **Maria Luisa Pinna Parpaglia:** Writing – review & editing, Investigation. **Alessandra Mollica:** Writing – review & editing, Investigation. **Maria Antonietta Amatori:** Writing – review & editing, Investigation. **Plamena Pentcheva:** Writing – review & editing, Investigation. **Giovanni Mario Careddu:** Writing – review & editing, Investigation. **Francesca Arru:** Investigation. **Chiara Gentile:** Writing – review & editing, Investigation. **Emanuela Seu:** Writing – review & editing, Investigation. **Alessia Pede:** Writing – review & editing, Validation, Conceptualization. **Claudia Tamponi:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Conceptualization. **Antonio Scala:** Writing – review & editing, Validation. **Simona Gabrielli:** Writing – review & editing, Methodology, Investigation. **Antonio**

Varcasia: Writing – review & editing, Validation, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization.

Ethical statement

All procedures involving animals were performed in accordance with relevant national and institutional guidelines for the care and use of animals. The study protocol was reviewed and approved by the Ethics Committee of the University of Sassari, Italy (protocol no. 54189, approved on 27 May 2025).

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.rvsc.2026.106280.

References

American Heartworm Society, 2024. Feline Guidelines for the Prevention, Diagnosis, and Management of Heartworm (*Dirofilaria immitis*) Infection in Cats. Revised 2024. https://www.heartwormsociety.org (accessed 10 October 2025).

Atkins, C.E., DeFrancesco, T.C., Coats, J.R., Sidley, J.A., Keene, B.W., 2000. Heartworm infection in cats: 50 cases (1985–1997). J. Am. Vet. Med. Assoc. 217, 355–358. https://doi.org/10.2460/javma.2000.217.355.

Atkins, C.E., Arther, R.G., Ciszewski, D.K., Davis, W.L., Ensley, S.M., Guity, P.S., Chopade, H., Hoss, H., Settje, T.L., 2008. Echocardiographic quantification of *Dirofilaria immitis* in experimentally infected cats. Vet. Parasitol. 158, 164–170. https://doi.org/10.1016/j.vetpar.2008.09.003.

Banca Dati Nazionale Animali da Compagnia Regione Autonoma della Sardegna, 2025. https://anagrafecanina.vetinfo.it/j6_siraaf/?p_reg_codice=200#/statistiche (accessed 03 March 2025).

Barriga, O.O., 1982. Dirofilariasis. In: Steele, J.H., Schultz, M.G. (Eds.), Handbook Series in Zoonoses, vol. 2. CRC Press Inc., Boca Raton, FL, pp. 93–110.

Berdoulay, P., Levy, J.K., Snyder, P.S., Pegelow, M.J., Hooks, J.L., Tavares, L.M., Gibson, N.M., Salute, M.E., 2004. Comparison of serological tests for the detection of natural heartworm infection in cats. J. Am. Anim. Hosp. Assoc. 40, 376–384. https://doi.org/10.5326/0400376.

Brianti, E., Panarese, R., Napoli, E., De Benedetto, G., Gaglio, G., Bezerra-Santos, M.A., Mendoza-Roldan, J.A., Otranto, D., 2022. *Dirofilaria immitis* infection in the Pelagie archipelago: the southernmost hyperendemic focus in Europe. Transbound. Emerg. Dis. 69, 1274–1280. https://doi.org/10.1111/tbed.14089.

Carbonara, M., Perles, L., Venco, L., Gabrielli, S., Barrs, V.R., Miró, G., Papadopoulos, E., Lima, C., Bouhsira, E., Baneth, G., Pantchev, N., Iatta, R., Mendoza-Roldan, J.A., Decaro, N., Schunack, B., Benelli, G., Otranto, D., 2025. *Dirofilaria* spp. infection in cats from the Mediterranean basin: diagnosis and epidemiology. Int. J. Parasitol. 55, 317–325. https://doi.org/10.1016/j.ijpara.2025.01.011.

Ciuca, L., Roman, C., Prisco, F., Miron, L., Acatrinei, D., Paciello, O., Maurelli, M.P., Vismarra, A., Cringoli, G., Rinaldi, L., 2020. First report of *Dirofilaria repens* infection in a microfilaraemic cat from Romania. Vet. Parasitol. Reg. Stud. Rep. 22, 100497. https://doi.org/10.1016/j.vprsr.2020.100497.

Corda, A., Corda, F., Secchi, V., Pentcheva, P., Tamponi, C., Tilocca, L., Varcasia, A., Scala, A., 2022. Ultrasonography of parasitic diseases in domestic animals: a systematic review. Animals 12, 1252. https://doi.org/10.3390/ani12101252.

DeFrancesco, T.C., Atkins, C.E., Miller, M.W., Meurs, K.M., Keene, B.W., 2001. Use of echocardiography for the diagnosis of heartworm disease in cats: 43 cases (1985–1997). J. Am. Vet. Med. Assoc. 218, 66–69. https://doi.org/10.2460/javma.2001.218.66.

Diakou, A., Soubasis, N., Chochlios, T., Oikonomidis, I.L., Tselekis, D., Koutinas, C., Karaiosif, R., Psaralexi, E., Tsouloufi, T.K., Brellou, G., Kritsepi-Konstantinou, M., Rallis, T., 2019. Canine and feline dirofilariosis in a highly enzootic area: first report of feline dirofilariosis in Greece. Parasitol. Res. 118, 677–682. https://doi.org/10.1007/s00436-018-6135-9.

Dillon, R., 1998. Clinical significance of feline heartworm disease. Vet. Clin. North Am. Small Anim. Pract. 28, 1547–1565. https://doi.org/10.1016/s0195-5616(98)50136-2.

European Society of Dirofilariosis and Angiostrongylosis (ESDA), 2017. Guidelines for Clinical Management of Feline Heartworm Disease. https://www.esda.vet (accessed 10 June 2025).

Favole, P., Cauduro, A., Opreni, M., Zanzani, S., Albonico, F., Manfredi, M., Cantile, C., Lorenzo, V., 2013. Epidural dirofilariosis in a paraparetic cat: case report of *Dirofilaria immitis* infection. J. Feline Med. Surg. 15, 1160–1164. https://doi.org/10.1177/1098612X13492740.

Foufopoulos, J., Roca, V., White, K., Pafilis, P., Valakos, E., 2016. Effects of island characteristics on parasitism in a Mediterranean lizard (*Podarcis erhardii*): a role of population size and island history? North-West. J. Zool. 13, 70–76.

Genchi, M., Rinaldi, L., Venco, L., Cringoli, G., Vismarra, A., Kramer, L., 2019. *Dirofilaria immitis* and *D. repens* in dog and cat: a questionnaire study in Italy. Vet. Parasitol. 267, 26–31. https://doi.org/10.1016/j.vetpar.2019.01.014.

Genchi, M., Kramer, L., Venco, L., Ciuca, L., Vismarra, A., 2023. How Italian practitioners manage dirofilariosis in dogs and cats? Vet. Parasitol. Reg. Stud. Rep. 46, 100934. https://doi.org/10.1016/j.vprsr.2023.100934.

Genchi, M., Escolar, I.R., García, R.M., Semeraro, M., Kramer, L.H., Colombo, L., Vismarra, A., 2024. *Dirofilaria immitis* in Italian cats and the risk of exposure by *Aedes albopictus*. Vector Borne Zoonotic Dis. 24, 151–158. https://doi.org/10.1089/vbz.2023.0097.

Giangaspero, A., Marangi, M., Latrofa, M.S., Martinelli, D., Traversa, D., Otranto, D., Genchi, C., 2013. Evidences of increasing risk of dirofilarioses in southern Italy. Parasitol. Res. 112, 1357–1361. https://doi.org/10.1007/s00436-012-3206-1.

Grillini, M., Frangipane di Regalbono, A., Tessarin, C., Beraldo, P., Cassini, R., Marchiori, E., Simonato, G., 2022. Evidence of *Dirofilaria immitis* in felids in North-Eastern Italy. Pathogens 11, 1216. https://doi.org/10.3390/pathogens11101216.

Knott, J., 1939. A method for making microfilarial surveys on day blood. Trans. R. Soc. Trop. Med. Hyg. 33, 191–196. https://doi.org/10.1016/S0035-9203(39)90101-X.

Kramer, L., Genchi, C., 2002. Feline heartworm infection: serological survey of asymptomatic cats living in northern Italy. Vet. Parasitol. 104, 43–50. https://doi.org/10.1016/s0304-4017(01)00602-1.

Lee, A.C., Atkins, C.E., 2010. Understanding feline heartworm infection: disease, diagnosis, and treatment. Top. Companion Anim. Med. 25, 224–230. https://doi.org/10.1053/j.tcam.2010.09.003.

Litster, A.L., Atwell, R.B., 2008. Feline heartworm disease: a clinical review. J. Feline Med. Surg. 10, 137–144. https://doi.org/10.1016/j.jfms.2007.09.007.

Louzada-Flores, V.N., Kramer, L., Brianti, E., Napoli, E., Mendoza-Roldan, J.A., Bezerra-Santos, M.A., Latrofa, M.S., Otranto, D., 2022. Treatment with doxycycline is associated with complete clearance of circulating *Wolbachia* DNA in *Dirofilaria immitis*-naturally infected dogs. Acta Trop. 232, 106513. https://doi.org/10.1016/j.actatropica.2022.106513.

Magi, M., Prati, M.C., Sebastiani, B., Bandecchi, P., Guberti, V., 2002. Seroprevalence of feline heartworm disease in Tuscany. Vet. Rec. 150, 415–416. https://doi.org/10.1136/vr.150.13.415.

Magnis, J., Lorentz, S., Guardone, L., Grimm, F., Magi, M., Naucke, T.J., Deplazes, P., 2013. Morphometric analyses of canine blood microfilariae isolated by the Knott's test enables species-specific diagnosis. Parasit. Vectors 6, 48. https://doi.org/10.1186/1756-3305-6-48.

McCall, J.W., Supakorndej, N., Ryan, W., Soll, M.D., 1995. Utility of an ELISA-based antibody test for detection of heartworm infection in cats. In: Soll, M.D., Knight, D.H. (Eds.), Proceedings of the Heartworm Symposium '95. American Heartworm Society, Batavia, IL, pp. 127–133.

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

Miller, M.W., Atkins, C.E., Stemme, K., Robertson-Plouch, C., Guerrero, J., 2000. Prevalence of exposure to *Dirofilaria immitis* in cats in multiple areas of the United States. Vet. Ther. 1, 169–175.

Nonnis, F., Atzeni, D., Cavallo, L., Gabrielli, S., Pombi, M., Cavallero, S., Bellini, I., Napoli, E., De Benedetto, G., Venco, L., Tamponi, C., Scala, A., Varcasia, A., 2025. Travelling safe? Risks associated to *Dirofilaria* spp. infection in dogs in a tourist destination. Curr. Res. Parasitol. Vector-Borne Dis. 8, 100298. https://doi.org/10.1016/j.crpvbd.2025.100298.

Oldach, M.S., Gunther-Harrington, C.T., Balsa, I.M., McLarty, E.M., Wakeman, K.A., Phillips, K.L., Honkavaara, J., Visser, L.C., Stern, J.A., 2018. Aberrant migration and surgical removal of a heartworm from the femoral artery of a cat. J. Vet. Intern. Med. 32, 792–796. https://doi.org/10.1111/jvim.15070.

Panarese, R., Iatta, R., Lia, R.P., Passantino, G., Ciccarelli, S., Gernone, F., Zatelli, A., Otranto, D., 2021. Dirofilarioses in two cats in southern Italy. Parasitol. Res. 120, 4247–4251. https://doi.org/10.1007/s00436-021-07127-6.

Pennisi, M.G., Tasker, S., Hartmann, K., Belák, S., Addie, D., Boucraut-Baralon, C., Egberink, H., Frymus, T., Hofmann-Lehmann, R., Hosie, M., Lloret, A., Marsilio, F., Thiry, E., Truyen, U., Möstl, K., 2020. Dirofilarioses in cats: European guidelines from the ABCD on prevention and management. J. Feline Med. Surg. 22, 442–451. https://doi.org/10.1177/1098612X20917601.

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. https://doi.org/10.1016/j.pt.2023.12.007.

Perles, L., Carbonara, M., Mendoza-Roldan, J.A., Venco, L., Gabrielli, S., Otranto, D., 2025. An indirect ELISA for the detection of antibodies against *Dirofilaria* spp. in cats. Parasit. Vectors 18, 16. https://doi.org/10.1186/s13071-024-06657-z.

Piché, C.A., Cavanaugh, M.T., Donoghue, A.R., Radecki, S.V., 1998. Results of antibody and antigen testing for feline heartworm infection at Heska® veterinary diagnostic laboratories. In: Seward, R.L. (Ed.), Recent Advances in Heartworm Disease: Symposium '98. American Heartworm Society, Batavia, IL, pp. 139–143.

Regione Autonoma della Sardegna, 2025. Dati regionali sul randagismo. https://www.pnrr.salute.gov.it/portale/caniGatti/dettaglioContenutiCaniGatti.jsp?lingua=italiano&id=280&area=cani&menu=abbandono (accessed 03 March 2025).

Robertson-Plouch, C.K., Dillon, A.R., Brawner, W.R., Guerrero, J., 2000. Prevalence of feline heartworm infections among cats with respiratory and gastrointestinal signs: results of a multicenter study. Vet. Ther. 1, 88–95.

Scala, A., Atzori, F., Varcasia, A., Garippa, G., Genchi, C., 2004. Canine filariosis in Sardinia: epidemiological updating (1998–2003). Atti Soc. It. Sci. Vet. 58, 120–121.

Scala, A., Pazzola, M., Giobbe, M., Sanna, G., Briguglio, P., Lutzu, M., Carta, A., Genchi, M., 2009. Is a heartworm preventive treatment also advisable in Sardinia?. In: 2nd Eur. Dir. Days 197.

Scala, A., Briguglio, P., Sanna, G., Pipia, A.P., Varcasia, A., Garippa, G., Genchi, M., Genchi, C., 2010. Feline heartworm (*Dirofilaria immitis*) infection in Sardinia. Parassitol 52, 257.

Simón, F., Siles-Lucas, M., Morchón, R., González-Miguel, J., Mellado, I., Carretón, E., Montoya-Alonso, J.A., 2012. Human and animal dirofilariasis: the emergence of a zoonotic mosaic. Clin. Microbiol. Rev. 25, 507–544. https://doi.org/10.1128/CMR.00012-12.

Simón, F., Diosdado, A., Siles-Lucas, M., Kartashev, V., González-Miguel, J., 2022. Human dirofilariosis in the 21st century: a scoping review of clinical cases reported in the literature. Transbound. Emerg. Dis. 69, 2424–2439. https://doi.org/10.1111/tbed.14210.

Thomas, W.P., Gaber, C.E., Jacobs, G.J., Kaplan, P.M., Lombard, C.W., Moise, N.S., Moses, B.L., 1993. Recommendations for standards in transthoracic two-dimensional echocardiography in the dog and cat. J. Vet. Intern. Med. 7, 247–252. https://doi.org/10.1111/j.1939-1676.1993.tb01015.x.

Traversa, D., Aste, G., Milillo, P., Capelli, G., Pampurini, F., Tunesi, C., Santori, D., Paoletti, B., Boari, A., 2010. Autochthonous foci of canine and feline infections by *Dirofilaria immitis* and *Dirofilaria repens* in central Italy. Vet. Parasitol. 169, 128–132. https://doi.org/10.1016/j.vetpar.2009.12.034.

Traversa, D., Morelli, S., Cassini, R., Crisi, P.E., Russi, I., Grillotti, E., Manzocchi, S., Simonato, G., Beraldo, P., Viglietti, A., De Tommaso, C., Pezzuto, C., Pampurini, F., Schaper, R., Frangipane di Regalbono, A., 2019. Occurrence of canine and feline extra-intestinal nematodes in Italy. Acta Trop. 193, 227–235. https://doi.org/10.1016/j.actatropica.2019.03.009.

Venco, L., Genchi, C., Genchi, M., Grandi, G., Kramer, L.H., 2008. Clinical evolution and radiographic findings of feline heartworm infection in asymptomatic cats. Vet. Parasitol. 158, 232–237. https://doi.org/10.1016/j.vetpar.2008.09.011.

Venco, L., Genchi, M., Genchi, C., Gatti, D., Kramer, L., 2011. Can heartworm prevalence in dogs be used as provisional data for assessing the prevalence of the infection in cats? Vet. Parasitol. 176, 300–303. https://doi.org/10.1016/j.vetpar.2011.01.013.

Venco, L., Marchesotti, F., Manzocchi, S., 2015. Feline heartworm disease: a Rubik's-cube-like diagnostic and therapeutic challenge. J. Vet. Cardiol. 17, S190–S201. https://doi.org/10.1016/j.jvc.2015.08.004.

Villanueva-Saz, S., Giner, J., Verde, M., Yzuel, A., González, A., Lacasta, D., Marteles, D., Fernández, A., 2021. Prevalence of microfilariae, antigen and antibodies of feline dirofilariosis infection (*Dirofilaria immitis*) in the Zaragoza metropolitan area, Spain. Vet. Parasitol. Reg. Stud. Rep. 23, 100541. https://doi.org/10.1016/j.vprsr.2021.100541.

Villanueva-Saz, S., Martínez, M.V., Alsina, S., Fernández, A., Gómez, Á., Verde, M., Ruiz, H., Lacasta, D., Alnassan, A.A., Trotta, M., Yzuel, A., Marteles, D., 2024. First report on natural infection by *Dirofilaria repens* in a cat in Spain. Vet. Res. Commun. 48, 1195–1201. https://doi.org/10.1007/s11259-023-10250-7.

Willems, A., Watteyne, S., Mertens, J., Gillis, M., Vandekerckhove, T.T.M., Swing, J.G., 1999. Phylogenetic analysis of the 16S rDNA of *Wolbachia*. FEMS Microbiol. Lett. 180, 279–286. https://doi.org/10.1111/j.1574-6968.1999.tb08807.x.