

## A cardiac and subcutaneous canine dirofilariosis outbreak in a kennel in central France

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**Abstract** – Canine dirofilarioses are nematode infections caused by two species of the genus *Dirofilaria*: *D. immitis* and *D. repens*. We describe here an outbreak of *D. immitis* and *D. repens* infection in military working dogs (MWDs) housed in a kennel in the Indre department (centre of France). Out of a total of 17 dogs, 6 (35.2%) tested positive for *D. immitis*, *D. repens* or both parasites. Infested dogs were treated and prophylactic measures were implemented for the entire kennel staff. To our knowledge, this is the first documented description of an outbreak of canine cardiopulmonary dirofilariosis in the center of France, unlike in the south of this country, where *D. immitis* and *D. repens* dirofilariosis are enzootic. In France, as mosquito vectors expand their territory and new non-native vectors are introduced, it is likely that the distribution area of these two diseases of domestic and wild carnivores will be wider and underestimated.

**Key words:** *Dirofilaria immitis*, *Dirofilaria repens*, Heartworm disease, PCR, Military working dog, France.

**Résumé** – Un foyer de dirofilariose canine cardiaque et sous-cutanée dans un chenil du centre de la France. Les dirofilarioses canines sont des infections à nématodes causée par deux espèces du genre *Dirofilaria*, *D. immitis* et *D. repens*. Nous décrivons ici un foyer d'infection à *D. immitis* et *D. repens* chez des chiens militaires hébergés dans un chenil dans le département de l'Indre (centre de la France). Sur un total de 17 chiens, 6 (35, 2 %) ont été testés positifs pour *D. immitis*, pour *D. repens* ou pour les deux parasites. Les chiens infestés ont été traités et des mesures prophylactiques ont été mises en place pour tout le personnel du chenil. À notre connaissance, il s'agit de la première description documentée d'un foyer de dirofilariose cardiopulmonaire canine dans le centre de la France, contrairement au sud du pays, où les dirofilarioses à *D. immitis* et *D. repens* sont enzootiques. En France, à mesure que les moustiques vecteurs élargissent leur territoire et que de nouveaux vecteurs non indigènes sont introduits, il est probable que la zone de distribution de ces deux maladies des carnivores domestiques et sauvages sera plus étendue et sous-estimée.

### Introduction

*Dirofilaria immitis* (Leidy, 1856) [23] and *Dirofilaria repens* Railliet & Henry, 1911 [33] are mosquito-borne filarioids (Nematoda: Onchocercidae) infecting wild and domestic mammals of different orders with canids as the predominant definitive hosts. Adult worms of *D. immitis* with a smooth cuticle (measuring between 12 cm and 30 cm in length) colonize the pulmonary arteries and right heart cavities, whereas adult worms of *D. repens*, with a finely striated cuticle, (measuring between 5 cm and 17 cm in length) are located in the subcutaneous tissues [11, 26, 28]. About 120 days after infection of the

mammalian host, the viviparous female can be fertilized and produces mobile embryos called microfilariae. Microfilariae (L1 stage) parasitize the blood until being ingested by the mosquito vector (Culicidae) during a blood meal taken on a microfilaremic host. Inside the vector, microfilariae first develop into larval stage 1 (L1), then molt into larval stage 2 (L2), and finally molt into third-stage larvae (L3), which is the infective stage. Clinically, *D. immitis* infection (canine heartworm disease) can remain unapparent for a long time. Symptoms are mainly dominated by right heart failure, ranging from exercise intolerance and fatigue to cardiac decompensation with swelling and possible acute pulmonary oedema [12, 26, 29]. In contrast, the infestation by *D. repens* often passes unnoticed. Dogs infected with *D. repens* microfilariae

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may sometimes develop subcutaneous nodules where adult worms encyst. Sometimes, the disease manifests with more symptoms, such as pruritus and skin changes [38].

Canine dirofilariosis has been known in France for a long time. It was in 1679 that Panhot highlighted heartworm in a dog native to the Dombes region (near Lyon) [30]. Over the last century, several studies have shown the importance of this parasitosis in the Mediterranean region [8, 18]. At the beginning of the 1980s, the infestation rate of military working dogs (MWDs) was high, particularly in Corsica and the Bouches-du-Rhône department [5, 8]. Out of 180 MWDs tested in 1988, 67 (37%) carried microfilariae in the blood, including 17 cases of *D. immitis*, 2 of *D. repens*, 15 mixed infestations and 3 doubtful ones [5]. A screening test carried out on 85 MWDs from northern France (Brittany and Normandy) was negative [6]. The MWDs with heartworm disease may lose their operational fitness, causing an operational impact for the Armed Forces. In fact, MWDs military dogs are used for the detection of explosive devices as well as to secure military sites. The eradication of canine heartworm disease among military dogs in south-eastern France was made possible by the implementation, for the first time in France, of chemoprophylaxis based on the use of ivermectin, completed by a conclusive trial on the efficacy of melarsomine in treating adult *Dirofilaria* [4]. The first demonstration of the long-term effectiveness of this protocol was made in 2015 by comparing the incidence rates of *D. immitis* infestation in MWDs from a kennel located in Corsica and in co-located civilian dogs (the civilian kennel was less than 15 km from the military kennel). The estimated prevalence in civilian dogs was 40.4% (19/47), while no cases were identified in MWDs [36]. Currently, canine dirofilariosis chemoprophylaxis is applied to all MWDs deployed on missions outside mainland France, as well as to MWDs from mainland France located in south-eastern France, during the period of vector activity (May–November). In the present study, we revealed the existence of an indigenous outbreak of *D. immitis* and *D. repens* infestation in the French department of Indre. To the best of our knowledge, this is the first documented description of an outbreak of canine cardiopulmonary dirofilariosis in this region of France.

## Materials and methods

### Dogs

In October 2018, we performed blood tests on the 17 apparently healthy dogs in the military kennel located near the city of Rosnay (46°42'47"N, 1°14'39"E), in Indre, central France. The commune is located in the "Parc naturel régional de la Brenne". The MWDs, including 11 Belgian Malinois and 6 German Shepherds, were all males aged 2–10 years, with a median of 5 years. The duration of the dogs' presence in the kennel ranged from 6 months to 8.5 years, with a median of 4 years. MWDs, including dogs from the Rosnay kennel, are purchased abroad, mainly in eastern European countries (Germany and Poland, in particular). They arrived at the kennel at about 2 years of age. They received regular veterinary care. From May to October, they benefit from a treatment against ectoparasites, but which has no repellent effect on mosquitoes. For the purpose

of the study, two blood samples of 4–5 mL volume were taken from each dog and collected from a dry tube and a citrated tube, respectively. Serum was collected after centrifugation (10 min, 3000 g). Each animal sampled was examined clinically.

### Ethics statement

Blood samples were collected in accordance with the requirements of the Animal Ethics Procedures of French veterinarians and with the consent of the owner of the animals (French Armed Forces).

### Direct detection of microfilariae

Modified Knott's staining was chosen to identify canine blood microfilaria [21, 25]. One millilitre of citrated blood was mixed with 9 mL of hemolyzing solution (2% acetic acid) in a 15 mL tube, followed by centrifugation for minutes at 500 rpm. The supernatant was removed; the sediment was stained with methylene-blue, transferred to a slide, and covered with a cover slip.

### Heartworm antigen detection

Heartworm antigen detection was performed using two rapid diagnostic serological tests marketed in France and targeting the antigen secreted by the adult female worm: (i) a WITNESS<sup>®</sup> *Dirofilaria* test (Zoetis, Lyon, France), based on immunomigration (RIM<sup>®</sup>) technology, and (ii) a DiroCHEK<sup>®</sup> test (Zoetis, Lyon, France), based on an enzyme-linked immunosorbent assay method known as a sandwich ELISA. Both tests provide rapid results, as well as high sensitivity (99% for WITNESS<sup>®</sup> *Dirofilaria* and 98% for DiroCHEK<sup>®</sup>) and specificity (94% for WITNESS<sup>®</sup> *Dirofilaria* and 96% for DiroCHEK<sup>®</sup>) [17].

### Molecular detection of filaria and the Wolbachia endosymbiont of filaria

DNA was extracted from 100 µL of citrated blood, after 4-hour digestion at 56 °C, using an equal volume of buffer G2 supplemented with 15% proteinase K. The extraction was performed using the Biorobot EZ1 System with the EZ1 DNA tissue kit (Qiagen, Courtaboeuf, France), in line with the manufacturer's instructions. DNA was eluted in a final volume of 100 µL and stored at –20 °C. All samples were screened for filaria and their *Wolbachia* complex, using the fast typing approach which consists in a pan-filarial 28S-based qPCR system, followed by a triplex COI-based system targeting *D. immitis*, *D. repens* and *Acanthocheilonema reconditum*, and the duplex *ftsZ*-based system, targeting specifically the *Wolbachia* endosymbiont of *D. immitis* and that of *D. repens* (Table 1) [22]. Once the specific filarial DNA has been revealed by the qPCR triplex, the sample is considered positive, and the detection of *Wolbachia* DNA confirms infection by *D. immitis* or *D. repens* related to this strain.

**Table 1.** Primers and probes used in this study.

System name	Primer & probe name	Sequences 5'-3'	Specificity	References
Pan-fil 28S qPCR-based system	qFil-28S-F	TTGTTTGAGATTGCAGCCCA	Filariae	[22]
	qFil-28S-P	6FAM-5'-CAAGTACCGTGAGGGAAAAGT-3'-TAMRA		
All-Wol 16S qPCR-based system	qFil-28S-R	GTTTCCATCTCAGCGGTTTC	Wolbachieae	
	all.Wol.16S.301-F all.Wol.16S.347-P	TGGAAGTACTGAGATACGGTCCAG 6FAM-5'-AATATTGGACAATGGGCGAA-3'-TAMRA		
Triplex TaqMan COI qPCR-based system	all.Wol.16S.478-R	GCACGGAGTTAGCCAGGACT	<i>Dirofilaria immitis</i>	
	Fil.COI.749-F	CATCCTGAGGTTTATGTTATTATTTT		
	D.imm.COI.777-P	6FAM-CGGTGTGGGATTGTTAGTG-TAMRA		
Duplex Wol-Diro ftsZ qPCR-based system	D.rep.COI.871-P	6VIC-TGCTGTTTTAGGTA CTCTGTTTGAG-TAMRA	<i>Dirofilaria repens</i>	
	A.rec.COI.866-P	Cy5-TGAATTGCTGTACTGGGA ACT-BHQ-3	<i>Acanthocheilonema reconditum</i>	
	Fil.COI.914-R	CWGTATACATATGATGRCCYCA	<i>Wolbachia</i> endosymbiont of <i>D. immitis</i> and <i>D. repens</i>	
Duplex HWs COI qPCR-based system	WDiro.ftsZ.490-F	AAGCCATTTTRGCTTYGAAGGTG	<i>D. immitis</i>	
	WDimm.ftsZ.523-P	6FAM-CGTATTGCAGAGCTCGGATTA-TAMRA		
	WDrep.ftsZ.525-P	6VIC-CATTGCAGAACTGGGACTGG-TAMRA		
Duplex HWs COI qPCR-based system	WDiro.ftsZ.600-R	AAACAAGTTTTGRTTTGGAATAACAAT	<i>Angiostrongylus vasorum</i>	
	Hw.COI.723-F	TCAGCATTTGTTTTGGTTTTT		
	D.imm.COI.777-P	6FAM-CGGTGTGGGATTGTTAGTG-TAMRA		
Pan-Nematoda primers 18S PCR-based system	A.vas.COI.813-P	6VIC-TGACTGGGAAGAAGGAGGTG-TAMRA	Nematoda	This study
	Hw.COI.950-R	GCASTAAAATAAGYACGAGWATC		
	Fwd.18S.631 Rwd.18S.1825r	TCGTCAATGCTGCGGTTAAA GGTTCAAGCCACTGCGATTAA		

## Sequencing analysis and phylogenetic genotyping of filaria

Samples harbouring a single DNA of filaria were subjected to sequencing analysis. The pan-Nematoda primers named Fwd.18S.631 & Rwd.18S.1825r (Table 1) were designed and customized to amplify an 1127–1155-bp fragment from the 18S rRNA gene. PCR reactions were carried out in a total volume of 50 µL, consisting of 25 µL of AmpliTaq Gold master mix, 18 µL of ultra-purified water DNase-RNase free, 1 µL of each primer and 5 µL of DNA template. The thermal cycling conditions were: incubation step at 95 °C for 15 min, 40 cycles of 1 min at 95 °C, 30 s at 54 °C for the melting temperature, and one and half minutes for the elongation time at 72 °C, followed by a final extension of 5 min at 72 °C. PCR amplification was performed in a Peltier PTC-200 model thermal cycler (MJ Research Inc., Watertown, MA, USA). The DNA generated through the PCR reaction was purified by filtration using a NucleoFast® 96 PCR DNA purification plate, and was then amplified using a BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA). The BigDye PCR products were purified on the Sephadex G-50 Superfine gel filtration resin prior to sequencing on the ABI Prism 3130XL. Nucleotide sequences were assembled and corrected using ChromasPro 2.0.0, then aligned against

close reference sequences of filarioids species, representative members of Onchocercidae available in GenBank. The alignment was performed using the ClustalW application within BioEdit v.7.2.5 [16]. The maximum likelihood phylogenetic tree was inferred on MEGA6 [37], based on the Kimura 3-substitution-type model [20].

## Results and discussion

Table 2 presents the results of the nine analyses carried out on the blood of 17 MWDs of the Rosnay kennel. For canine dirofilariosis, we observed 35.2% (6/17) positive dogs. The Knott test conducted on dog No. 3 revealed the presence of *D. immitis* microfilariae. DNA and *Wolbachia* complex qPCRs tests as well as the two serological tests gave positive results. Five dogs had mixed infestation with *D. immitis* and *D. repens* (dogs Nos. 2, 7, 8, 10 and 13). *Dirofilaria repens* DNA was identified in dogs Nos. 2, 10 and 13. Furthermore, both *Wolbachia* genotypes known to be associated with *D. immitis* and *D. repens* were also detected in the same samples. Two samples (Nos. 2 and 13) provided positive results with both serological tests. One dog (No. 7) was positive for both *D. immitis* and *D. repens* microfilariae by the Knott test, the PCR test for both *Dirofilaria* and *Wolbachia* DNA, and by

**Table 2.** Screening for dirofilariosis in a military kennel in the Indre department (central France).

Dog number (No.)	Breed*	Age (year)	Kennel presence time (year)	Parasitological diagnosis: Knott test	Serological screening		Molecular detection of filarial DNA using the qPCR Pan-Filaria 28S	Genotyping: 18S rRNA gene	Molecular identification of filarial species using a COI Triplex qPCR-based system			Molecular identification of <i>Wolbachia</i> genotypes using <i>ftsZ</i> duplex qPCR-based system		Diagnosis
					Witness® Dirofilaria	DiroCHEK®			<i>Dirofilaria immitis</i>	<i>Dirofilaria repens</i>	<i>Acanthocheilonema reconditum</i>	<i>Wolbachia</i> endosymbiont of <i>D. immitis</i>	<i>Wolbachia</i> endosymbiont of <i>D. repens</i>	
1	BSM	10	8.5	Neg.	Neg.	Neg.	Neg.	NE**	Neg.	Neg.	Neg.	Neg.	Neg.	Healthy dog
2	BSM	7	6	<i>D. repens</i>	Pos.	Pos.	Pos.	<i>D. repens</i>	Neg.	Pos.	Neg.	Pos.	Pos.	Occult heartworm and subcutaneous dirofilariosis
3	GS	6	5	<i>D. immitis</i>	Pos.	Pos.	Pos.	<i>D. immitis</i>	Pos.	Neg.	Neg.	Pos.	Neg.	Heartworm disease
4	BSM	6	5	Neg.	Neg.	Neg.	Neg.	NE	Neg.	Neg.	Neg.	Neg.	Neg.	Healthy dog
5	BSM	6	5	Neg.	Neg.	Neg.	Neg.	NE	Neg.	Neg.	Neg.	Neg.	Neg.	Healthy dog
6	BSM	6.5	5	Neg.	Neg.	Neg.	Neg.	NE	Neg.	Neg.	Neg.	Neg.	Neg.	Healthy dog
7	GS	6	4	<i>D. immitis</i> + <i>D. repens</i>	Neg.	Pos.	Pos.	NE	Pos.	Pos.	Neg.	Pos.	Pos.	Heartworm and subcutaneous dirofilariosis
8	BSM	4.5	3	Neg.	Neg.	Neg.	Pos.	NE	Pos.	Pos.	Neg.	Pos.	Pos.	Occult heartworm and subcutaneous dirofilariosis
9	GS	3.5	2.5	Neg.	Neg.	Neg.	Neg.	NE	Neg.	Neg.	Neg.	Neg.	Neg.	Healthy dog
10	BSM	4	2	<i>D. repens</i>	Neg.	Neg.	Pos.	<i>D. repens</i>	Neg.	Pos.	Neg.	Pos.	Pos.	Occult heartworm and subcutaneous dirofilariosis
11	BSM	3	2	Neg.	Neg.	Neg.	Neg.	NE	Neg.	Neg.	Neg.	Neg.	Neg.	Healthy dog
12	GS	3	2	Neg.	Neg.	Neg.	Neg.	NE	Neg.	Neg.	Neg.	Neg.	Neg.	Healthy dog
13	GS	4	1.5	<i>D. repens</i>	Pos.	Pos.	Pos.	<i>D. repens</i>	Neg.	Pos.	Neg.	Pos.	Pos.	Occult heartworm and subcutaneous dirofilariosis
14	GS	2.5	1.5	Neg.	Neg.	Neg.	Neg.	NE	Neg.	Neg.	Neg.	Neg.	Neg.	Healthy dog
15	BSM	3.5	1	Neg.	Neg.	Neg.	Neg.	NE	Neg.	Neg.	Neg.	Neg.	Neg.	Healthy dog
16	BSM	2	0.5	Neg.	Neg.	Neg.	Neg.	NE	Neg.	Neg.	Neg.	Neg.	Neg.	Healthy dog
17	BSM	2	0.5	Neg.	Neg.	Neg.	Neg.	NE	Neg.	Neg.	Neg.	Neg.	Neg.	Healthy dog

\* BSM: Belgian shepherd malinois, GS: German shepherd.

\*\* NE: Not evaluated.





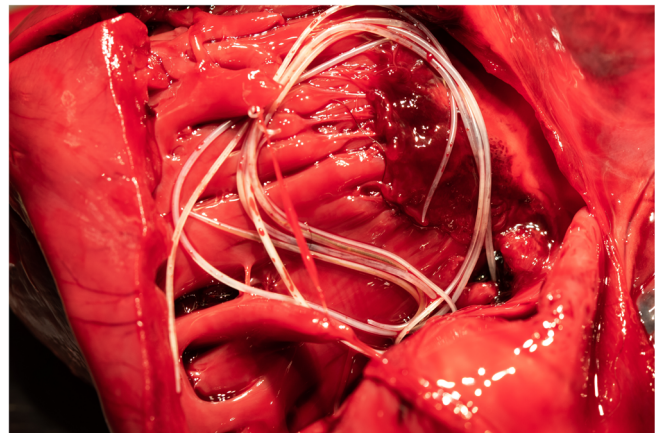
**Figure 1.** Molecular phylogenetic analysis of the 18S rRNA gene, using the maximum likelihood method based on the Kimura 3-substitution-type model.

ELISA; whereas, it was negative by the immunomigration test. Finally, dog No. 8 was positive for *D. immitis* and *D. repens* and *Wolbachia* DNA; and for both serological tests. All samples were free of *A. reconditum* infection.

Four partial sequences of the 18S rRNA gene have been successfully generated from samples identified as positive for a single DNA of filaria. Phylogenetic analysis revealed that dog No. 3 was infected with *D. immitis*, having 100% identity with *D. immitis* isolated from foxes in France (Marseille) (MK673809, MK673810) and 99.9% with that isolated from dogs in Japan (AB973231). Three *D. repens* sequences were 100% identical both to each other and to *D. repens* isolated from humans in Japan (Fig. 1).

Dog No. 7 died suddenly in November 2018 (1 month after blood sampling for our study) because of a stomach dilation-torsion. The necropsy performed on this dog revealed the presence in the right heart of four females and two males of *D. immitis* (Fig. 2). The heart was not dilated.

It is known that heartworm can occur as an occult infestation, resulting in the presence of at least one mature female (or male) of *D. immitis*, without circulating microfilariae. Occult infestations occur in several situations, including monosexual infestation by male or female worms only, infertility of female worms, low infestation levels and/or destruction of microfilariae due to the host's immune response [35]. In our study, the proportion of occult infestation among dogs infected by *D. immitis* was 66.6% (4/6). The detection of occult infections is usually based on adult worm antigen testing [24]. However, in areas endemic for both *D. immitis* and *D. repens*, the reliability of these tests decreases, due to two main factors: (i) cross-reaction of rapid diagnostic tests between *D. immitis* and other parasites, including *D. repens*, and (ii) the immunosuppressive capacity



**Figure 2.** Heartworm (*Dirofilaria immitis*) in the right ventricle of dog No. 7.

of *D. repens* over *D. immitis* microfilariae, which hinders diagnosis based on the detection of blood microfilariae [14]. Adulticide treatment of *D. immitis* dirofilariasis, according to the protocol recommended by the American Heartworm Society, was effective against *D. repens* [28]. The approach combining the specific detection of molecular markers of *D. immitis* and *D. repens* showed 100% and 99.3% sensitivity and specificity, respectively. The reliability of the method is not reduced, even in case of occult dirofilariasis possibly accompanied by infestation by other filaroid species like *Acanthocheilonema reconditum*, leading to false positives following cross-reactions of rapid detection tests for *D. immitis* antigens [24]. This is coherent with the results obtained in our study, where occult heartworm

infection associated with circulating microfilariae of *D. repens* was observed in 60% (3/5) of *Dirofilaria* spp. coinfecting dogs, of which only 40% (2/5) were positive for *D. immitis* antigen.

Canine filarial infections have increased significantly in recent years [35]. This trend is the consequence of the increase in the range of vectors, as well as the introduction of infected dogs, reservoirs of parasites, into ecosystems favourable to the emergence of secondary indigenous outbreaks. The geographical distribution of heartworm disease and subcutaneous dirofilariasis in France is not precisely known. However, several studies reported that *D. repens* is more endemic and spreads more rapidly than *D. immitis* within northern and eastern Europe [2, 3, 14, 31, 36]. These differences in the epidemiology of the two parasite species could be explained by the fact that in areas where *D. repens* is widespread, the progression of *D. immitis* would be hindered, and *vice versa* [14]. Outbreaks of *D. immitis* have also been reported in western France, they concerned hunting dogs living in kennels near ponds [6, 7]. In our study, the military kennel is located in an infertile swampy area near seven ponds. The local ecosystem is highly favourable to the development of dirofilariasis vectors. *Aedes (Stegomyia) albopictus*, more commonly known as the “tiger mosquito”, has been introduced into metropolitan France from Italy and is now present throughout the south of the country, as well as in regions further north, including the Paris region and the department of Indre since 2017 [27]. This highly invasive and anthropophilic mosquito is known to be a competent vector of dirofilariasis parasites and to be implicated in the transmission of dirofilariasis in the studied area and most likely contributed to the infection of the military dogs considered in this study [36]. In southern Italy, the worrying increase in the proportion of mosquitoes infected by *D. immitis* among *A. albopictus* populations is associated with an increased risk of infestation of dogs with this parasite [15]. An integrated approach to control dirofilariasis vectors and to reduce infection sources and reservoirs for the parasite should be implemented in these areas. In the highly endemic area, the multi-modal prophylactic strategy, consisting in the administration of macrocyclic lactones and the application of repellents effective against mosquitoes, appears to be a tailored strategy.

A wide range of mammalian hosts, including humans and cats, can be infected by both *D. immitis* and *D. repens*, resulting from their low host specificity. Human infestation by *D. immitis* results in a pulmonary form (nodules), the most severe but less frequent form, which is found mainly in southern Europe (Italy, Spain, Greece, etc.) [13]. In France, most cases of human dirofilariasis caused by *D. immitis* are reported in endemic regions, such as Corsica and the Bouches-du-Rhône department [9, 32]. In humans, like in dogs, *D. repens* causes subcutaneous filariasis [1, 19].

We report here for the first time, an outbreak of canine dirofilariasis (*D. repens* and *D. immitis*) in the Rosnay military kennel, with a health risk for military personnel and military dogs in this area. In late 2018, after the detection of several infected dogs in the kennel, the following management measures were implemented: all dogs infected by *D. immitis* and cases of mixed infection ( $N = 6$ ), except one that died before the treatment was administered (dog No. 7), received adulticide

(melarsomine), combined with larvicide treatment (ivermectin), doxycycline and glucocorticosteroids (prednisone), according to the treatment protocol recommended by the American Heartworm Society [28]. During treatment, a restriction on physical activity was prescribed. This treatment eliminated *D. repens* and *D. immitis* larvae, as well as *Wolbachia* complex and the existing susceptible larvae. Moreover, all infected dogs were treated with insecticidal repellent effective against mosquitoes during the period of vector activity (from May to November in mainland France). The aim was to prevent secondary cases and outbreaks, in the presence of competent vectors in the area where the military kennel is located.

## Conclusions

The epidemiology of dirofilariasis infections is complex and has even been related to the concept of the epizootic, represented by the multiple interactions between climate, environment, animals, humans and parasites [34]. As these are zoonoses, physicians and veterinarians must be informed of the risks of transmission in the regions, and especially the local biotopes [10]. We suggest that epidemiological investigations of vectors and canine dirofilariasis be implemented in areas at risk of exposure. Moreover, we suggest implementation of prevention against *D. immitis* and *D. repens* infection in dogs, using macrocyclic lactones [35], in combination with a repellent effective against mosquitoes (permethrin or deltamethrin-based products), as recommended for heartworm prevention in the affected and surrounding areas [28].

## Competing interests

The authors declare that they have no competing interests.

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