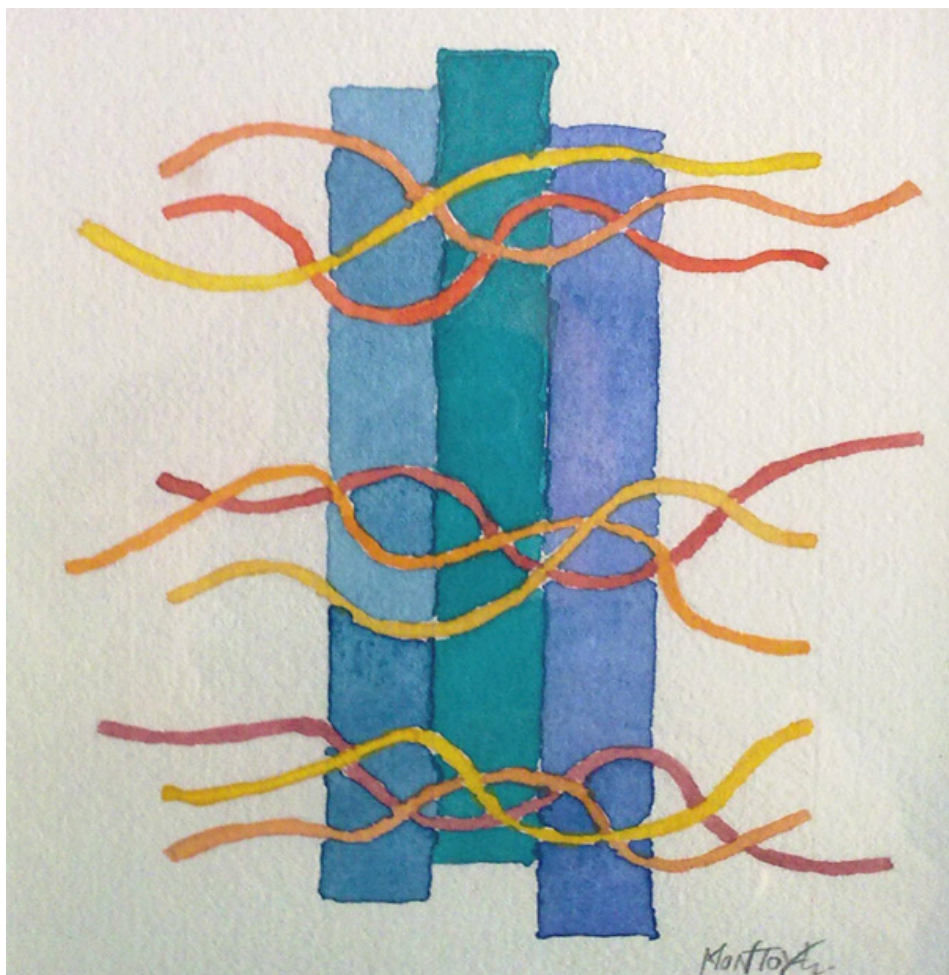




UNIVERSIDAD DE LAS PALMAS DE GRAN CANARIA  
Facultad de Ciencias de la Salud



**TESIS DOCTORAL**  
**INFESTACIÓN POR *DIROFILARIA IMMITIS***  
**EN LAS ISLAS CANARIAS**



**José Alberto Montoya Alonso**  
Las Palmas de Gran Canaria, 24 de septiembre de 2015



**Anexo I**

**DR. OCTAVIO LUIS PEREZ LUZARDO SECRETARIO DEL  
DEPARTAMENTO DE CIENCIAS CLINICAS DE LA  
UNIVERSIDAD DE LAS PALMAS DE GRAN CANARIA,**

**CERTIFICA,**

Que el Consejo de Doctores del Departamento en su sesión de fecha 22 de septiembre de 2015, tomó el acuerdo de dar el consentimiento para su tramitación, a la tesis doctoral titulada "INFESTACIÓN POR DIROFILARIA IMMITIS EN LAS ISLAS CANARIAS" presentada por el doctorando D. José Alberto Montoya Alonso y dirigida por el Dr. D. Fernando Lorenzo Simón Martín.

Y para que así conste, y a efectos de lo previsto en el Artº 6 del Reglamento para la elaboración, defensa, tribunal y evaluación de tesis doctorales de la Universidad de Las Palmas de Gran Canaria, firmo la presente en Las Palmas de Gran Canaria, a 22 de septiembre de dos mil quince.







UNIVERSIDAD DE LAS PALMAS DE GRAN CANARIA  
Facultad de Ciencias de la Salud



DEPARTAMENTO DE CIENCIAS CLÍNICAS

PROGRAMA DE DOCTORADO EN  
MICROBIOLOGIA

**TESIS DOCTORAL  
INFESTACIÓN POR *DIROFILARIA IMMITIS*  
EN LAS ISLAS CANARIAS**

**DOCTORANDO**

**José Alberto Montoya Alonso**

**DIRECTOR**

**Fernando Lorenzo Simón Martín**  
**Universidad de Salamanca**

Las Palmas de Gran Canaria, 24 de septiembre de 2015





**Fernando Simón Martín**, Doctor en Biología y Catedrático de Parasitología del Departamento de Biología Animal, Parasitología, Ecología, Edafología y Química Agrícola de la Universidad de Salamanca,

**INFORMA:**

Que D. José Alberto Montoya-Alonso, Doctor en Veterinaria, ha realizado, bajo mi dirección y asesoramiento, la presente Tesis Doctoral titulada: “Infestación por *Dirofilaria immitis* en las islas Canarias”, que a mi juicio reúne las condiciones y calidad científica necesarias para su presentación y defensa, para optar al título de Doctor por la Universidad de Las Palmas de Gran Canaria.

Lo que firmo, para que surta los efectos a que haya lugar, en Salamanca a quince de septiembre de 2015.

Fdo.: Prof. Fernando Simón Martín. USAL

*Las ilustraciones de este trabajo han sido cedidas por mi hermano el Dr. Carlos Montoya Alonso*

*Trabajo maquetado por Ernest Julio, Multimédica Ediciones Veterinarias*

A mi madre Pili (Q.E.P.D.) que siempre estuvo orgullosa de mí

A Cande, Alberto y Nacho con todo mi cariño

A toda mi familia por lo que me quiere y aguanta

A Elena Carretón por ser como es





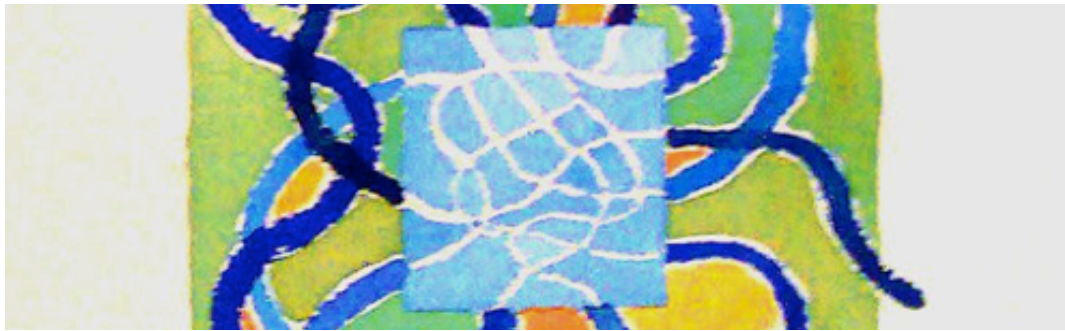
## AGRADECIMIENTOS

Quiero expresar mi sincero agradecimiento a:

- Toda mi familia por su continuo cariño y apoyo.
- Todos mis amigos, de todo el mundo, que no se cansan de aguantarme.
- Todos mis estudiantes por su reconocimiento, gratitud y por servirme de continuo estímulo, durante tanto años.
- La profesión veterinaria y la Universidad que forman parte de mi forma de ser y vivir.
- Todos los colaboradores de esta tesis por su dedicación y amistad. En particular al entrañable profesor Fernando Simón.
- Los colegas, clínicas y hospitales veterinarios por lo que nos alientan, nos reconocen y confían en nosotros.
- Las instituciones públicas y privadas, que financian nuestras investigaciones, por su confianza y apoyo.
- Los profesionales y centros médicos y sanitarios por toda su colaboración, comprensión y ayuda recibida.
- Nuestros pacientes y sus propietarios, por las satisfacciones que nos proporcionan, por todo lo que nos aguantan y por ser comprensivos con nuestros errores.
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## INTRODUCCIÓN

La dirofilariosis cardiopulmonar (*Dirofilaria immitis*) es una enfermedad de distribución mundial, siendo las áreas endémicas las zonas templadas, tropicales y sub-tropicales del planeta, debido, fundamentalmente, a las necesidades climáticas de los insectos vectores. Sin embargo, las regiones más frías, con veranos cálidos, grandes ríos y/o lagos y extensas zonas que regadío, ofrecen también lugares idóneos para el desarrollo de los mosquitos. La incidencia de la enfermedad se ha incrementado drásticamente en los últimos años y se ha extendido de los países tropicales y sub-tropicales a las zonas templadas, debido, con mucha probabilidad, al aumento de movilidad de los animales reservorios entre otras causas.

En los últimos años se han realizado, en los diferentes países, numerosos estudios epidemiológicos, que han demostrado que la dirofilariosis es una enfermedad emergente. Tiene una distribución mundial, fundamentalmente por las zonas templadas del planeta; las prevalencia más elevadas se han registrado en América, África, Polinesia, Australia y Japón.

En Europa, la incidencia más alta se registra en los países mediterráneos con una prevalencia global de un 10%. Sin embargo, destacan algunas zonas del norte de Italia, sur de Francia y España con con infestaciones superiores al 20%. España, por tanto, se encuentra entre las regiones enzoóticas de dirofilariosis.

La prevalencia en España es elevada sobre todo en la costa del sur peninsular tanto mediterránea como atlántica, así como en las islas Canarias. Es precisamente en el archipiélago Canario, situado en el paralelo 28 frente a las costas atlánticas del continente africano, donde la tasa de incidencia alcanza valores más elevados; se considera zona hiperendémica y los veterinarios clínicos incluyen la dirofilariosis como primera posibilidad diagnóstica en perros con síntomas cardiopulmonares.

Por ello, el objetivo de este trabajo ha sido conocer la incidencia de la infestación por *Dirofilaria immitis* en animales domésticos y seroprevalencia en el hombre en el archipiélago Canario, así como su evolución temporal desde 1984 hasta la actualidad.

Nuestra relación con las filarias comenzó hace muchos años. Siendo estudiante de 4º curso, en la Facultad de Veterinaria de Madrid, asistí a mi primera necropsia de una perra, de raza pastor alemán, que había muerto de un síndrome de vena cava, producido por los múltiples gusanos adultos de *D. immitis*, que invadían su corazón y sus vasos. Debo reconocer que la escena me produjo gran impresión.

Al llegar a la isla de Gran Canaria, a principio de los 90, y gracias a muchos compañeros, pero principalmente a un magnífico veterinario canario como es Manolo Morales Doreste, empecé a trabajar con los perros parasitados de filaria y a reconocer sus síntomas, realizar diagnósticos y a aplicar las medidas terapéuticas disponibles en esa época.

En 1998 publicamos el primer estudio epidemiológico de prevalencia de esta enfermedad en Gran Canaria, considerando las características isoclimáticas de la isla y sus particularidades geográficas. Este trabajo ha sido referente de muchos estudios epidemiológicos posteriores.

Por aquella época, el querido y entrañable Prof. Zoilo González Lama (Q.E.P.D.) me propuso matricularme de doctorado en su Departamento de Ciencias Clínicas e iniciar esta Tesis Doctoral. Siempre me estuvo animando a seguir con el estudio de la dirofilariosis en Canarias, pero desgraciadamente no pudo ver la obra concluida.

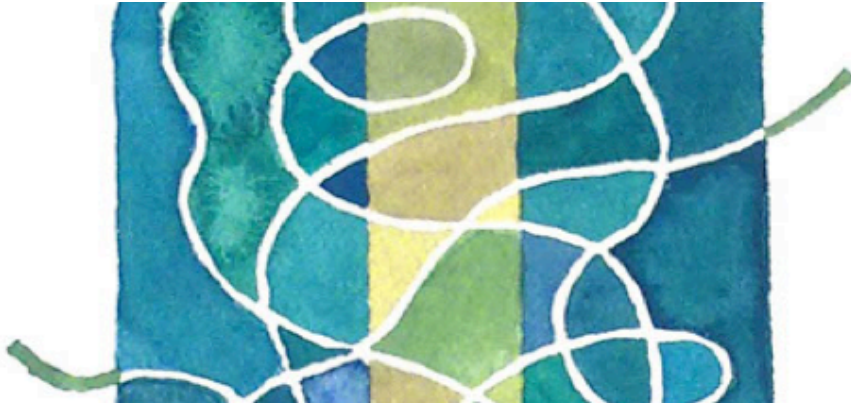
Más tarde conocí al Prof. Fernando Simón Martín, salmantino, amigo y catedrático de Parasitología de la Facultad de Farmacia de la Universidad de Salamanca y uno de los más importantes investigadores mundiales de dirofilariosis que, con mucho cariño, me invitó a investigar con su grupo y a dedicarme con más intensidad a trabajar con la enfermedad del gusano del corazón.

Al grupo de medicina interna (cardio-respiratorio) de la Facultad de Veterinaria de Las Palmas de Gran Canaria, se incorporó hace unos años, con una beca de formación del personal investigador, Elena Carretón Gómez. La Dra. Carretón, Elena, es una bilbaína-canaria infatigable que nunca se desalienta, ni deja de trabajar con las filarias y sus pacientes, y una magnífica compañera. Lógicamente esto supuso un incremento exponencial de nuestra actividad investigadora.

Necesariamente tuvimos que ampliar nuestros contactos con grupos multidisciplinares nacionales e internacionales, especialistas en esta zoonosis parasitaria (Profesores: Morchón, Genchi, Kramer, Guerrero, Mc.Call, Kartashev, Grandi, González-Miguel, Kozek, Siles-Lucas, Venco...) a los que agradecemos su apoyo y sin los que hubiera sido imposible avanzar en nuestro trabajo.

Más becarios, estudiantes y proyectos han ido reforzando al equipo y nuestras investigaciones. Actualmente formamos parte activa de la European Dirofiliariasis Society (EDIS) y nuestro trabajo va adquiriendo reconocimiento internacional, con más de 30 artículos específicos de alto impacto, libros, monografías, proyectos, premios...

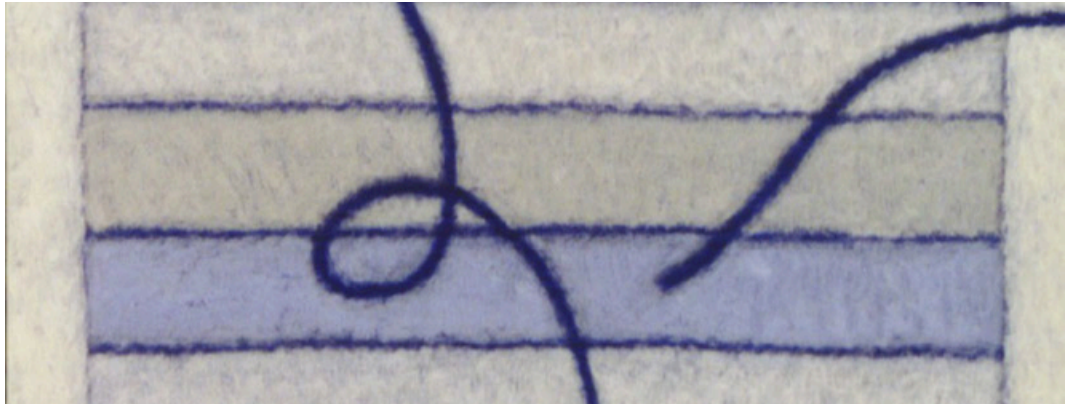
Así, nuestro trabajo clínico e investigador, de varios años, sobre la enfermedad en Canarias y su importancia zoonótica ha quedado reflejando de manera sencilla, en esta tesis en formato de compendio de publicaciones.



## OBJETIVOS

1. Determinar la prevalencia de *Dirofilaria immitis* en perros de la isla de Gran Canaria los años 1994,1995 y 1996.
2. Conocer la prevalencia de *D. immitis* en perros de la isla de Tenerife y sus variaciones en función de los diferentes isoclimas de la isla (años 2002-2003).
3. Valorar las variaciones de la incidencia de *D. immitis* en perros, en la isla de Gran Canaria, entre 2000 y 2008 y determinar si el podenco canario constituye un reservorio natural de la enfermedad.
4. Conocer la seroprevalencia de *D. immitis* en la población humana y su relación con la prevalencia en perros en la isla de Gran Canaria (año 2008).
5. Estudiar la incidencia de la zoonosis en perros, gatos y humanos en las diferentes zonas isoclimáticas de Gran Canaria, y evaluar la relación entre las prevalencias de las diferentes especies (año 2010).
6. Determinar la incidencia de la dirofilariosis cardiopulmonar en pequeños animales en todo el archipiélago Canario (años 2014-15).





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### **Human and animal dirofilariasis: the emergence of a zoonotic mosaic**

Simón F, Siles-Lucas M, Morchón R, González-Miguel J, Mellado I, Carretón E, Montoya-Alonso JA

*Clinical Microbiology Reviews*, 25 (3): 507-544, 2012





# Human and Animal *Dirofilariasis*: the Emergence of a Zoonotic Mosaic

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## INTRODUCTION

A Lombard noble named Francesco Birago made the first known reference to canine filariae in the 17th century by describing the presence of adult *Dirofilaria immitis* worms inside the hearts of his hunting dogs, although he erroneously identified them as larvae of another parasitic worm, possibly *Dyoctophyma renale* (49). Currently, *dirofilariasis* is understood as a group of parasitoses caused by species of the genus *Dirofilaria* transmitted by vectors. Among all *Dirofilaria* species, the most relevant are *D.*

*immitis* and *D. (Nochtiella) repens* due to their severe pathological effects and their high prevalence and incidence. *D. immitis* pro-

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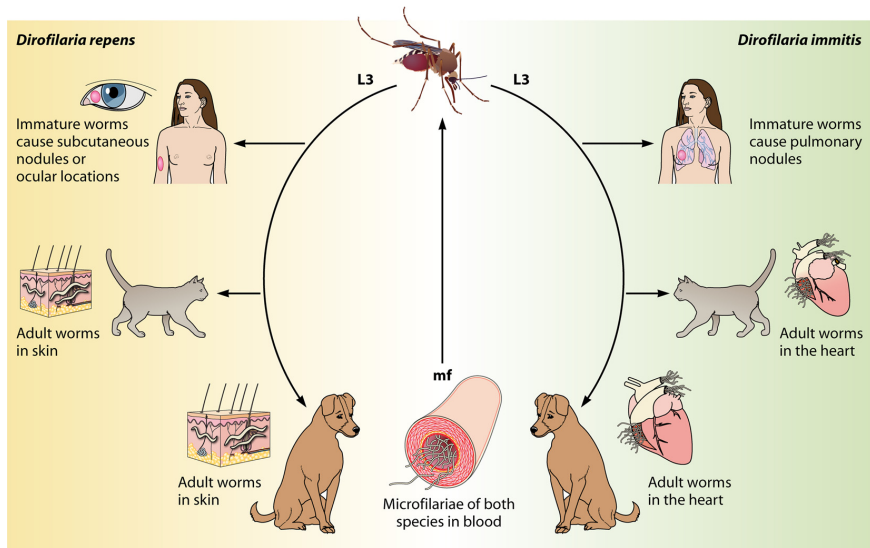


FIG 1 Biological life cycles of *D. immitis* and *D. repens*. mf, microfilaria.

duces both canine and feline cardiopulmonary dirofilariasis, whereas *D. repens* causes both canine and feline subcutaneous dirofilariasis. In addition, *D. immitis* and *D. repens* are responsible for human pulmonary and subcutaneous/ocular dirofilariasis, respectively, throughout the world (324, 391). Therefore, these infections represent a zoonotic mosaic, which in practice includes two main filarial species that have adapted to canine, feline, and human hosts to various degrees. In each of these hosts, *D. immitis* and *D. repens* exhibit specific developmental patterns, each with distinct biological and clinical implications. At the same time, both *D. immitis* and *D. repens* are themselves hosts to symbiotic bacteria of the genus *Wolbachia*, the study of which has resulted in a profound shift in the understanding of filarial biology, the mechanisms of the pathologies that they produce in their hosts, and issues related to the treatment of this parasitosis. Additionally, the involvement of vectors in the parasite life cycle makes dirofilariasis transmission and distribution susceptible to global climate change, and rates have undergone rapid and significant changes in defined geographic regions in recent years. Despite advances in our knowledge of *D. immitis* and *D. repens* and the pathologies that they inflict on different hosts, dirofilariasis remains a priority subject of study in veterinary medicine nearly 400 years after its discovery. Therefore, there is greater interest in and attention to the increasing incidence and severity of human dirofilariasis cases. In consideration of all of these issues, we review human and animal dirofilariasis here, including the basic morphology, biology, protein composition, and metabolism of *Dirofilaria* species; the climate and human behavioral factors that influence distribution dynamics; disease pathology; the host-parasite relationship; the mechanisms involved in parasite survival; the immune response and pathogenesis; and the clinical management of human and animal infections.

## ESSENTIAL FEATURES OF THE BIOLOGY OF *DIROFILARIA* SPECIES

### Life Cycle

The life cycle of *Dirofilaria* species comprises a definitive vertebrate host and a vector (Fig. 1). Both *D. immitis* and *D. repens* demonstrate poor vertebrate host specificity given that they can infect numerous mammalian species (34). Among mammalian hosts, they are best adapted to domesticated and wild dogs, which function as reservoirs. Humans and cats are less suitable hosts (261), in which parasite development is dramatically modified compared with the patterns in dogs. The vectors are females of various mosquito species of the *Culicidae* family (75).

**Development in the definitive host.** During a blood meal, mosquitoes deposit a hemolymph on the wound, which carries infectious “larvae 3” (L3) stage larvae that penetrate the host’s skin on their own (435). The molt from L3 to L4 occurs soon after *D. immitis* infection, between 3 and 12 days postinfection (d.p.i.), and the subsequent molt, which produces preadult worms, takes place between 50 and 70 d.p.i. The first preadult worms arrive in the pulmonary artery and right ventricle of canine hearts at between 70 and 85 d.p.i. and reach sexual maturity at 120 d.p.i. Adult *D. immitis* worms have a filiform appearance, with females measuring 250 to 300  $\mu\text{m}$  in length and 1 to 1.3 mm in diameter and males measuring 120 to 200  $\mu\text{m}$  in length and 0.7 to 0.9 mm in diameter (252) (Fig. 2). Females start producing the first larval stage (microfilariae) between 6 and 9 months postinfection (m.p.i.) (261). Microfilariae that live in the bloodstream are 290 to 330  $\mu\text{m}$  in length and 5 to 7  $\mu\text{m}$  in diameter, with a straight tail and a spindle-shaped cephalic extremity. Adults can live over 7 years, and microfilariae live as long as 2 years (435). Some infected dogs do not harbor microfilariae in the blood, resulting in occult or amicrofilaremic infections, possibly due to factors such as the

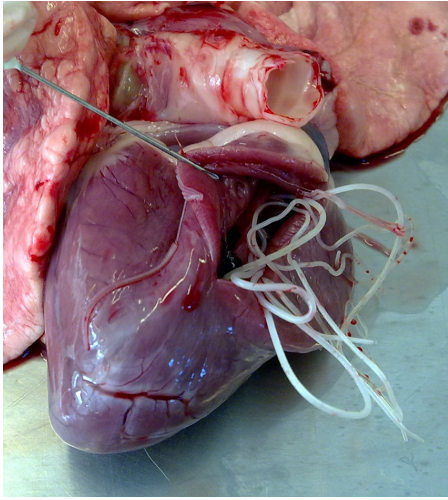


FIG 2 Male and female adult worms of *D. immitis* in the heart of a dog.

aging of female worms, single-gender worm infections, and/or host immune responses (388). In cats, the maturation of adult *D. immitis* worms extends up to 8 m.p.i.; these worms are shorter than those found in dogs, with a shorter life expectancy (approximately 2 years maximum), and they generally do not produce microfilariae. When microfilaremia does occur in this host, it is transient and of a low intensity (252, 261).

Adult *D. repens* worms generally take residence in the subcutaneous tissues of definitive hosts, although they can also be found in the abdominal cavity and within connective muscular fasciae (148), where they achieve sexual maturity at 6 to 9 m.p.i. (252). *D. repens* worms are smaller than *D. immitis* worms: females are 100 to 170 mm in length and 4.6 to 6.3 mm in diameter, and males are 50 to 70 mm in length and 3.7 to 4.5 mm in diameter (252). Like those of *D. immitis*, *D. repens* microfilariae reside in the bloodstream, measuring between 350 and 385  $\mu\text{m}$  in length and 7 to 8  $\mu\text{m}$  in diameter, with a curved tail and rounded cephalic extremity (435). The finding that cats harbor *D. repens* microfilariae in their blood suggests that feline hosts may serve as reservoirs for this species (419).

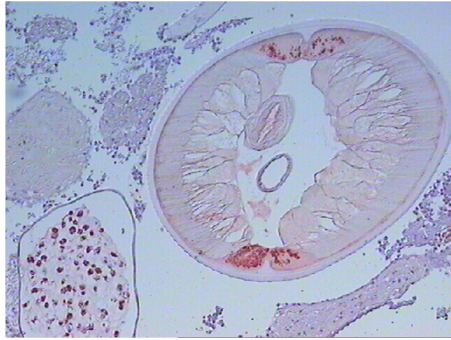
Humans are not suitable hosts for *Dirofilaria* species, as demonstrated by their deviation from the developmental patterns described above. Immature *D. immitis* worms can reach a branch of the human pulmonary artery, triggering an inflammatory response that destroys the worms, occasionally resulting in pulmonary nodules (391). *D. repens* worms cause subcutaneous nodules and can reach the ocular region in human patients (321). Both species can infect anatomical regions other than those described above but only incidentally (328, 423).

**Development in mosquito vectors.** *D. immitis* and *D. repens* microfilariae are ingested by mosquitoes during a blood meal on an infected host. Within approximately 24 h, the ingested microfilariae reach the Malpighian tubules from the digestive tract, where they molt to the L2 larval form within approximately 8 to 10 d.p.i. and from L2 to L3 approximately 3 days afterwards. The environmental temperature is the key factor that determines the

length of the L3 developmental period in mosquitoes. The infective L3 larvae migrate to the mouthparts of the vector, where they reside until the next blood meal. L3 larvae are approximately 1 mm in length and grow to 1.5 mm in the subcutaneous tissues of definitive hosts upon inoculation (252). The invasion of Malpighian tubules and migration to the mouthparts of the parasite are critical for mosquito survival. The vectors limit the number of larvae that can progress to L3 via antigenic recognition and humoral and cellular defense mechanisms (83). An antimicrobial polypeptide, defensin, has been identified in mosquito hemolymph inoculated with *D. immitis* L3 larvae. In addition, *D. immitis* vectors exhibit the capacity for the melanization and encapsulation of L3 larvae. Melanin is a pigment produced in hemocytes via the conversion of tyrosine to dopamine through hydroxylation by a phenoloxidase (181). Tyrosine, the limiting factor for the entire process, can be obtained exogenously or can be derived from phenylalanine by phenylalanine hydroxylase (PAH), which is an enzyme that is required for a complete melanization reaction in vectors (185). Melanization concludes with the formation of a membrane-like structure on the external zone of the cellular capsule (83). The melanization efficiency varies across species and among members of the same species due to differences in phenoloxidase activity, which is more robust during the initial 14 days of the life of the mosquito (91, 232). Other mechanisms and structures contribute to the destruction of larvae, such as the buccopharyngeal armature (cibarial armature), which can damage microfilariae during a blood meal; the secretion of molecules that lyse the epicuticle of worms; and blood coagulation, which traps microfilariae in the digestive tract of mosquitoes, thereby impeding their passage to the Malpighian tubules (74). Mosquitoes' susceptibility and resistance to infection are genetically determined and controlled by a gender-linked recessive allele, with separate genes coding for susceptibility and resistance to *D. immitis* and *D. repens* (74). Genetic differences that influence transmission may also exist within a single species: a study performed in a western region of Spain where *D. immitis* is endemic found *D. immitis* larvae only in the H1 haplotype of *Culex pipiens* but not in haplotypes H2 and H3 (286).

#### Role of *Wolbachia* Symbiotic Bacteria in Development and Biology of *Dirofilaria* Species

The first intracellular bacterium-like bodies in filariae were found in *D. immitis* (174). Later research demonstrated the bacterial nature of these bodies (266) and their presence in other species of filariae, such as *Onchocerca volvulus* (210). Two decades later, studies using electron microscopy and molecular techniques demonstrated that these bacteria belong to the order *Rickettsiales* (alpha-2-proteobacteria) and the genus *Wolbachia* (399). These bacteria have been also found in other organisms, including hexapods, crustaceans, and chelicerates. *Wolbachia* bacteria are intracellular, are observed in isolation or in clusters (81, 133), and seem to have developed a symbiotic relationship with these and other organisms, including filariae of the family Onchocercidae, of which *D. immitis* and *D. repens* are members (134). Studies of the antibiotic treatment of filaria-infected hosts and genome sequencing of *Wolbachia* have provided information about the nature of the interactions between bacteria and filariae and the molecules involved (140, 454). Those studies suggested that *Wolbachia* bacteria are involved in molting and embryogenesis of filariae (28), whereas filariae contribute amino acids for bacterial



**FIG 3** Immunohistochemical positive reaction (red) against the *Wolbachia* surface protein (WSP) reveals the presence of symbiotic bacteria in the hypodermal cords of a *D. repens* adult worm. (Courtesy of L. H. Kramer, University of Parma, Parma, Italy.)

growth (140). *Wolbachia* are transmitted maternally, are found in all individuals at all filarial developmental stages, and are particularly abundant in larvae that develop in vertebrate hosts (L3 and L4), in the hypodermal cords of adults of both genders (Fig. 3), and in the genital organs of females. These findings suggest that symbiotic bacteria are essential for larval development in vertebrate hosts and for the long-term survival of adult worms (263). *Wolbachia* was recently found in new species of filariae from the family Onchocercidae in various organs, such as somatic gonads (epithelial layer) and the intestinal wall, suggesting that the bacterium-filaria relationship is far more complex and diverse than previously estimated (135).

#### Classical and Proteomic Approaches to the Study of Proteins in *Dirofilaria* spp.

Databases of protein sequences from the National Center for Biotechnology Information (NCBI) contain approximately 136 records for *D. immitis*, a very limited amount of genetic information compared with those for other nematodes (for example, 23,329 records for *Brugia malayi*). Several studies in the 1980s and 1990s identified single molecules in the different developmental stages of *D. immitis* and some of their characteristics. In adult worms, a significant proportion of the molecules identified were acidic polypeptides that ranged in size from 82 kDa to >200 kDa and were susceptible to various collagenases (381). Later, the antigenic repertoires of L2 and L3 larvae were found to be similar to but different from that of L4 larvae (382). Among the differences, a 35-kDa polypeptide was identified and characterized as an immunodominant surface antigen that is present in L3 larvae but not in larvae at subsequent developmental stages (340). A nonimmunogenic 6- to 10-kDa glycolipid was also identified (381). Both the 35-kDa and 6-kDa molecules are shed from the surface of L3 larvae during the early days of development *in vivo* and *in vitro*, and the released material is not replenished (184).

Later, several other proteins from *D. immitis* were identified, cloned, and characterized, among which the heat shock protein p27 is noteworthy due to its potential role in the host-parasite relationship. p27, to which repair functions during development have been attributed, is localized to the hypodermis of L3 and L4 larvae and adults (236). Additionally, various enzymes with redox

potential have been described, including peroxiredoxins in somatic extracts and excretory-secretory (E/S) products from adults and microfilariae (88, 463) and glutathione peroxidase in adult worms and L4 larvae (428), which is a precursor of a neutrophil chemotactic factor (320). Two nuclear receptors, Di-nh-7 and Di-RXR-1, the latter being related to cell proliferation, differentiation, and apoptosis (99, 385), have also been reported. A chitin synthase (175) and an ivermectin-sensitive glutamate-gated chloride channel subunit (458) have also been characterized.

In addition to those single-molecule identification approaches, proteomics were later applied to the study of dirofilarial proteins. Together with mass spectrometry, proteomics allowed the simultaneous identification of numerous proteins, comprising 39 proteins from *D. immitis* and 15 from *D. repens*, many of which were represented by several isoforms (157, 158, 313). These proteins belong primarily to four functional groups, including metabolic enzymes, enzymes with redox or detoxification potential, and molecules involved in motility and the stress response (Table 1). The most represented enzymes are those involved in energy metabolism, eight of which participate in anaerobic glycolysis in *D. immitis*, among them enolase, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and lactate dehydrogenase. Five proteins with redox potential and four with roles in stress responses, including various heat shock proteins, were also identified. Many of these molecules have antioxidant and detoxification properties and have been linked to the parasites' capacity to neutralize reactive oxygen species released by macrophages and neutrophils (158). Many of the proteins within these groups have also been found in *D. repens* albeit in a lower number than in *D. immitis* (Table 1). Antigenic extracts of *D. immitis* contain an abundance of glycosylated molecules, although glycosylated residues are not exposed on the surface of intact worms. Glycosylated residues contain primarily mannose, glucose, fucose, *N*-acetylglucosamine, and *N*-acetylgalactosamine. *N*-Acetylgalactosamine has been found in an unusual terminal via a  $\beta$ -linkage to the sequence GalNac $\beta$ GlcNac $\beta$ Man-R, which is a structure similar to *N*-union glycoproteins in mammals albeit with significant differences, such as the lack of both sialic acid and galactose (195, 196, 272).

#### Energy Generation and Molting in *Dirofilaria* Species

**Nutrient uptake and energy metabolism.** Although the molecules involved in *Dirofilaria* energy metabolism could provide excellent targets for future therapeutic or control measures, few studies have been conducted on this topic. Recently, studies of the relationship between filariae and *Wolbachia* and some proteomic analyses of worms have begun to yield valuable information about the molecules involved in the various metabolic processes that are key to the survival and development of *D. immitis* and *D. repens*.

The routes for nutrient acquisition are different in the several developmental stages of *Dirofilaria* spp. Adult *D. immitis* worms have a functional digestive tract, whereas microfilariae do not, and the digestive tract of L3 larvae is nonfunctional in vectors but becomes functional in the vertebrate host (33, 70). In adult worms, nutrient uptake is performed in the digestive tract, in which erythrocytes have been found (33), and the cuticle *in vivo*, whereas the transcuticular route is the main means for the uptake of low-molecular-weight molecules *in vitro* (207) (Fig. 4). Transcuticular uptake is a selective process, as demonstrated by the finding that worms acquire D-glucose and some precursors of nucleic acids but not L-glucose, sucrose, or thymidine via this route.

TABLE 1 Proteins of *D. immitis* and *D. repens* identified by mass spectrometry<sup>a</sup>

GenBank accession no.	Protein	Species	No. of isoforms	Mascot score	Biological process
<i>D. immitis</i> proteins					
CAA34719	Actin	<i>Caenorhabditis elegans</i>	4	202–450	Cell motility
P30162	Actin-1	<i>Onchocerca volvulus</i>	1	321	Cell motility
P30163	Actin-2	<i>Onchocerca volvulus</i>	2	245–367	Cell motility
NP_508842	ACTin family member (act-4)	<i>Caenorhabditis elegans</i>	3	146–180	Cell motility
ID4X_A	Chain A, crystal structure of Mg-ATP actin	<i>Caenorhabditis elegans</i>	1	355	Cell motility
AAF32254	Heat shock protein 70	<i>Wuchereria bancrofti</i>	4	285–679	Stress response
CAA61152	Small heat shock protein	<i>Brugia pahangi</i>	4	86–116	Stress response
AAB08736	Small heat shock protein p27	<i>Dirofilaria immitis</i>	1	203	Stress response
CAA48632	OV25-1 protein	<i>Onchocerca volvulus</i>	5	123–232	Stress response
XP_001896281	Enolase	<i>Brugia malayi</i>	6	115–427	Glycolysis
AAB52600	Fructose-bisphosphate aldolase	<i>Onchocerca volvulus</i>	7	112–248	Glycolysis
XP_001899850	Glyceraldehyde-3-phosphate dehydrogenase	<i>Brugia malayi</i>	4	100–349	Glycolysis
AAV33247	Phosphoglycerate mutase	<i>Onchocerca volvulus</i>	2	152–158	Glycolysis
XP_001891892	Phosphoglycerate kinase	<i>Brugia malayi</i>	3	104–149	Glycolysis
EDP29666	Glucose phosphate isomerase	<i>Brugia malayi</i>	2	128–133	Glycolysis
XP_001897269	Triosephosphate isomerase	<i>Brugia malayi</i>	4	163–448	Glycolysis
XP_001900208	Lactate dehydrogenase	<i>Brugia malayi</i>	1	113	Anaerobic glycolysis
XP_001900957	Fumarase	<i>Brugia malayi</i>	3	91–200	Aerobic metabolism
A8NLA3	Hypothetical FAD-dependent oxidoreductase	<i>Brugia malayi</i>	3	123–153	Electron transport
XP_001901495	Nucleoside diphosphate kinase	<i>Brugia malayi</i>	2	136–139	Nucleotide metabolism
XP_001897743	Oxidoreductase, aldo/keto reductase family protein	<i>Brugia malayi</i>	1	130	Redox process
AAC24752	Transglutaminase precursor	<i>Dirofilaria immitis</i>	3	109–115	Redox homeostasis
CAE11787	Protein disulfide isomerase	<i>Brugia malayi</i>	1	234	Redox homeostasis
AAC38831	Thioredoxin peroxidase	<i>Dirofilaria immitis</i>	2	118–124	Redox homeostasis
P52033	Glutathione peroxidase, Di29 precursor	<i>Dirofilaria immitis</i>	1	121	Oxidative stress response
CAA73325	Glutathione transferase	<i>Brugia malayi</i>	1	99	Detoxification
AAC47233	Cyclophilin Ovcyp-2	<i>Onchocerca volvulus</i>	2	118–231	Protein folding
AA799423	Peptidylprolyl isomerase	<i>Taenia solium</i>	1	119	Protein folding
XP_001902628	Bmcp-2	<i>Brugia malayi</i>	1	144	Protein folding
EDP37909	Rab GDP dissociation inhibitor alpha	<i>Brugia malayi</i>	1	596	Protein transport
BAA96354	Phosphatidyl-ethanolamine-binding protein	<i>Dirofilaria immitis</i>	2	129–230	Signal transduction
XP_001899662	OV-16 antigen precursor	<i>Brugia malayi</i>	1	97	Signal transduction
AAZ42332	G protein $\beta$ subunit	<i>Caenorhabditis remanei</i>	1	134	Signal transduction
AAF37720	Galectin	<i>Dirofilaria immitis</i>	5	132–203	Immune response
XP_001899521	Disorganized muscle protein 1	<i>Brugia malayi</i>	3	358–439	Cell adhesion
Q27384	Pepsin inhibitor Dit33 precursor	<i>Dirofilaria immitis</i>	4	178–379	
XP_001670614	Hypothetical protein CBG05397	<i>Caenorhabditis briggsae</i>	4	85–140	
AAD11968	P22U	<i>Dirofilaria immitis</i>	2	510–582	
BAA02004	Neutrophil chemotactic factor precursor	<i>Dirofilaria immitis</i>	2	124	
<i>D. repens</i> proteins					
ABH11671	Actin	<i>Chara contraria</i>	3	128–145	Cell motility
XP_001898433	Actin 2	<i>Brugia malayi</i>	2	485–541	Cell motility
NP_508842	ACTin family member (act-4)	<i>Caenorhabditis elegans</i>	8	132–274	Cell motility
XP_001898461	Troponin family protein	<i>Brugia malayi</i>	1	101	Cell motility
XP_001895017	Heat shock 70-kDa protein C precursor	<i>Brugia malayi</i>	2	124–138	Stress response
XP_001896281	Enolase	<i>Brugia malayi</i>	5	87–226	Glycolysis
AAB52600	Fructose-bisphosphate aldolase	<i>Onchocerca volvulus</i>	6	101–129	Glycolysis
XP_001899850	Glyceraldehyde-3-phosphate dehydrogenase	<i>Brugia malayi</i>	3	101–125	Glycolysis
XP_001901359	Proteasome subunit alpha type 7-1	<i>Brugia malayi</i>	1	100	Protein catabolism
AAC24752	Transglutaminase precursor	<i>Dirofilaria immitis</i>	3	104–137	Redox homeostasis
AAC47233	Cyclophilin Ovcyp-2	<i>Onchocerca volvulus</i>	1	112	Protein folding
BAA96354	Phosphatidyl-ethanolamine-binding protein	<i>Dirofilaria immitis</i>	1	110	Signal transduction
XP_001898507	Immunoglobulin I-set domain-containing protein	<i>Brugia malayi</i>	1	103	Signal transduction
XP_001900812	Galectin	<i>Brugia malayi</i>	3	98–191	Immune response
XP_001899521	Disorganized muscle protein 1	<i>Brugia malayi</i>	3	124–328	Cell adhesion

<sup>a</sup> The Mascot score is the score given as  $S = -10 \times \log(P)$ , where  $P$  is the probability that the observed match would be a random event. Mascot score values above 80 are considered significant ( $P < 0.05$ ). FAD, flavin adenine dinucleotide.



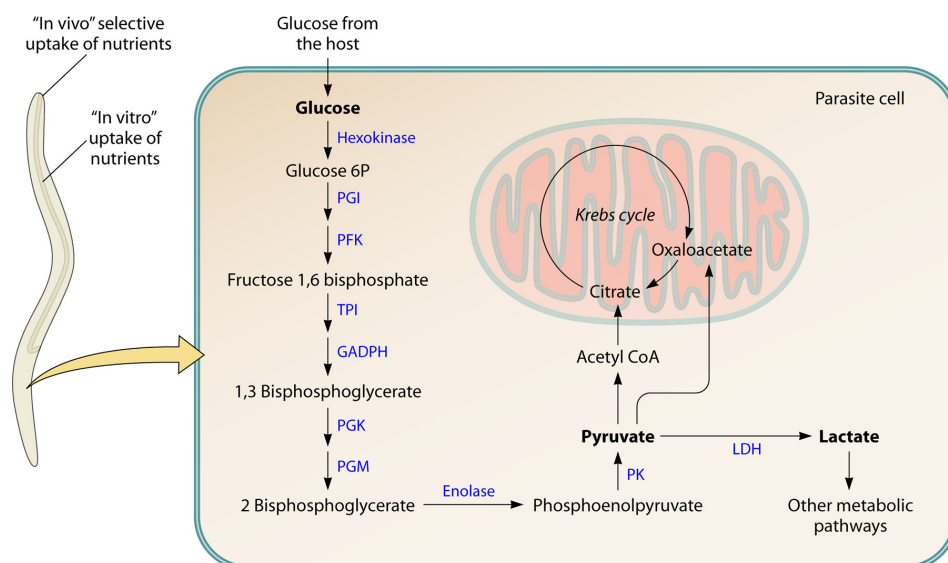


FIG 4 Nutrient uptake and energy metabolism of *D. immitis*. There is a selective uptake of nutrients through both the digestive tract and the cuticle in microfilariae and adult worms. The main route for energy generation is anaerobic glycolysis with lactate as a main end product. Enzymes involved in glycolysis identified in *D. immitis* are indicated. PGI, phosphoglucose isomerase; PFK, phosphofructokinase; TPI, triosephosphate isomerase; GADPH, glyceraldehyde-3-phosphate dehydrogenase; PGK, phosphoglycerate kinase; PGM, phosphoglycerate mutase; PK, pyruvate kinase; LDH, lactate dehydrogenase; bisP, bisphosphate; CoA, coenzyme A.

In microfilariae, nutrient acquisition is exclusively transcuticular, and only glucose, some amino acids, and RNA precursors are taken up (8, 188). Regarding protein digestion, significant protease activity has been observed in the subcutaneous tissue and, to a lesser extent, in the intestine and other anatomical sites, such as the testes, germ cells, uterine wall, and fertilized eggs of adult worms (249, 456). These findings suggest that protease activity is related not only to nutrition but also to other processes, such as spermatogenesis in males and the production of microfilariae in females (373). In addition, L3 and L4 larvae from *D. immitis* secrete proteases that lyse proteins in subcutaneous tissues, doubling the collagen lysis capacity of L3 larvae during molting, in a process essential for the completion of their life cycle (355).

With respect to energy metabolism, it is known that the majority of the glucose absorbed by *D. immitis* is metabolized via different pathways, and only trace amounts are accumulated as glycogen (188), showing the presence of hexoses, pentoses, and their derivatives in extracts of the parasite (430). Adult *D. immitis* worms are regarded as homolactic fermenters because lactate is the main (or only) product of monosaccharide lysis produced by the worms (Fig. 4), indicating that their metabolism is mainly anaerobic (207). The ratio of pyruvate kinase activity to phosphoenolpyruvate carboxykinase activity is an indicator of the relative importance of anaerobic versus aerobic metabolism (33). This ratio is significantly elevated in adult *D. immitis* worms, confirming the dominantly anaerobic nature of their energy metabolism (64). Many glycolytic enzymes have been found in *D. immitis* (70). The presence of several isoforms of some of those glycolytic enzymes has been demonstrated for both *D. immitis* and *D. repens* (158, 313), which could provide parasites with an increased ability

to thwart any host interference in the energy generation processes required for parasite survival. In contrast, the aerobic metabolism of carbohydrates predominates in microfilariae (188), and oxygen is required for motility although apparently not for survival (207).

The relevance of mitochondrial respiration in *Dirofilaria* and other filariae is not fully known, because although they possess all of the enzymes required for the Krebs cycle, no malate dehydrogenase activity has been detected, and levels of aconitase and isocitrate dehydrogenase activities are low. The low levels of activity or the lack of these enzymes suggests that the Krebs cycle is not essential for the metabolism of adult worms (70).

**Molting.** With each of the four molts that occur in nematodes, a new cuticle develops, with a characteristic structure and collagen profile (322). Structural changes of the cuticle along the various *D. immitis* developmental stages have been studied. In L3 and L4 larvae, the cuticle has a trilaminar appearance and exhibits a complex structure. Several proteases, such as transglutaminase and cysteine protease, assist in the release of the old cuticle during the L3-to-L4 molt (87, 354), indicating that inhibitors of these enzymes could halt molting and the subsequent development of adult worms within vertebrate hosts. The cuticle of the adult worms shows a cortical envelope divided into an outer layer and an inner layer. The internal layer is similar to a trilaminar cellular membrane, whereas the external layer consists of an amorphous osmophilic layer. In the space between the layers, fibers reminiscent of collagen surround keratin-like material, the arrangement of which confers flexibility to the worms, which is required for their survival. The epicuticle is lined with a thin glycocalyx (1, 48, 233, 234, 265). Nematode molting is regulated by ecdysteroid hormones, which play a similar role in insects (31). A receptor for

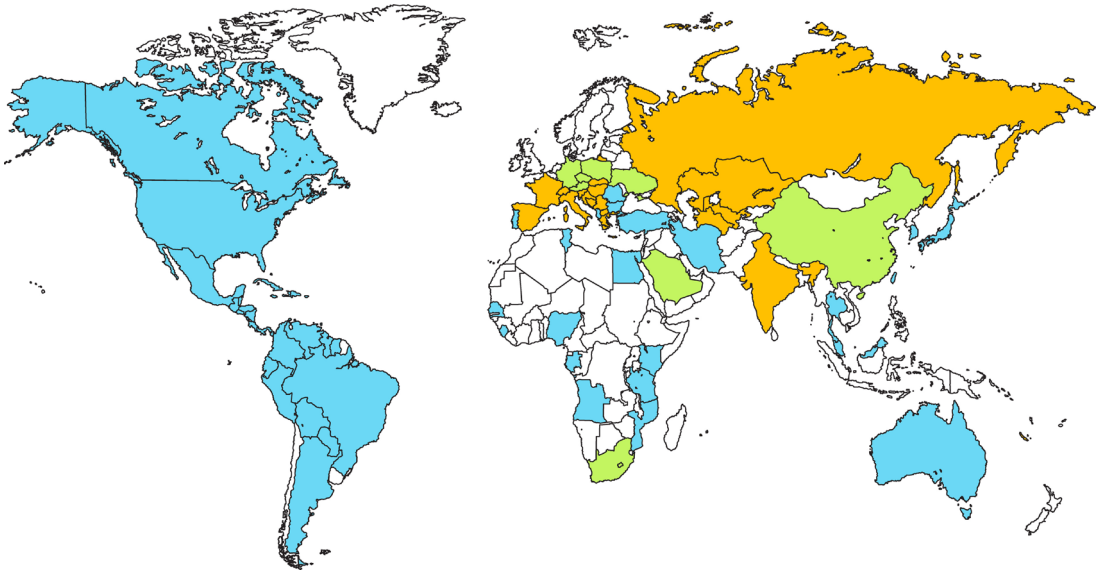


FIG 5 Current geographical distribution of canine dirofilariasis. Blue, *D. immitis* infections; green, *D. repens* infections; orange, presence of both species.

20-hydroxyecdysone (DiEcR) that shares multiple biochemical features with a similar receptor in insects has been identified in *D. immitis* and characterized. This type of receptor is found in filariae whose life cycle includes a mosquito vector, but it has not been found in either nematode species that do not require vector-based transmission, e.g., the plan parasite *Meloidogyne incognita*, or free-living nematode species such as *Caenorhabditis elegans* (386). Both ecdysone and 20-hydroxyecdysone have been found free in the reproductive tract and body wall and as conjugates in the reproductive system and the digestive tract of *D. immitis* (93). Nevertheless, the ability of nematodes to synthesize such molecules has not been demonstrated to date (32). In this respect, *Wolbachia* provides the heme group to filariae, which is an essential component of cytochrome P450, required for the biosynthesis of steroidal hormones, among others (305). Since the elimination of symbiotic bacteria leads to the inhibition of the molting process in filariae, future research may provide precise information regarding how *Wolbachia* participates in the biosynthesis of steroidal hormones related to molting, helping worms with synthesis through their above-mentioned molecular contributions. Recently, a new actor in the molting process in nematodes, Di-nhr-6, an ortholog of the E75 (NR1D3) gene from *Drosophila melanogaster*, involved in molt control and metamorphosis, was characterized in *D. immitis*. This gene encodes canonical protein receptors, is developmentally regulated, and is expressed in a gender-specific manner in adults (100).

#### PREVALENCE AND DISTRIBUTION DYNAMICS

*D. immitis* affects wild and domestic canines and felines and human populations in tropical and temperate regions throughout the world, whereas *D. repens* is exclusive to the Old World. Most of the available epidemiological information originates from a limited number of countries in which these illnesses have been con-

sidered both veterinary and medical concerns for decades. Nevertheless, with increasing frequency, data are being generated in countries where dirofilariasis was not previously documented or for which information was limited. A comparison of the historical epidemiological data in a 10-year period shows that changes in the distribution and prevalence of dirofilariasis are occurring throughout the world. These changes could be partially attributed to the growing interest of the scientific community in dirofilariasis, especially with respect to human infections, and to climate change, which has increased the range of specific vectors of *Dirofilaria* spp. in some regions.

#### Canine Dirofilariasis

**The Americas.** *D. immitis* affects canine populations in most countries of the Americas except Chile, where it has not been found despite epidemiological surveys, and French Guiana and Uruguay, where studies have not been conducted (170, 220, 278, 442, 445) (Fig. 5). Dirofilariasis is spread throughout the United States, with a 1 to 12% prevalence rate (226). Initially, it was enzootic in states along the Atlantic Coast; the Mississippi, Missouri, and Ohio rivers; and the Gulf Coast, where the prevalence can reach 48.8% (58, 229, 261), and new foci have been documented in central and Western states (Wyoming, Utah, Montana, California, Oregon, and Washington) (147). In Central and South America, dirofilariasis prevalence rates range from 20% to 42% in cities on the Gulf Coast of Mexico, from 20.4% to 63.2% in the Caribbean (the Bahamas, Curaçao, Cuba, the Dominican Republic, and Puerto Rico), from 2.3% to 45% in Brazil, and from 5% to 74% in Argentina (6, 211, 218, 220, 251, 442). In Bolivia, dirofilariasis has been found in dogs and in wild canids (67, 136) and has been found only in dogs in the Galapagos Islands of Ecuador (228). Prevalence rates are much lower in regions with colder climates, such as Canada, where the reported mean prevalence rate is 0.24%

(402). However, prevalences in cold areas can reach up to 8.4%, such as in southern Ontario (204).

Periodic surveys have shown an increasing trend in the prevalence of dirofilariasis. Studies performed in the United States from 2002 to 2005 demonstrated that the incidences of cardiopulmonary dirofilariasis had increased in 30 states, decreased in 17 states, and remained steady in 3 states, with a total increase of over 5,000 infected animals during this period (170). In 2008, diagnosed cases of cardiopulmonary dirofilariasis were reported in every U.S. state (306), although transmission in Alaska is not well documented (226). In South America, Labarthe and Guerrero (220) found increasing prevalence rates in Colombia (from 4.8% to 8.4%) and Argentina (from 3.5% to 5.1%) and declining rates in Brazil (from 7.9% to 2%) based on comparisons of the earliest and most recently published reports from those countries.

**Europe.** As described above, the epidemiological profile of canine dirofilariasis in Europe is characterized by the presence of *D. immitis* and *D. repens*, the coexistence of both species in some countries, and their expansion within countries where they are traditionally endemic and toward northern and central Europe (most noticeably *D. repens*) (Fig. 5). Among countries with historical records on dirofilariasis, only Portugal reported the presence *D. immitis*, with the highest reported prevalence rate (30%) for the island of Madeira (11). *D. immitis* is present throughout most of Spain (although some regions have not been surveyed), with prevalence rates of 33 to 40% in the Canary Islands and some humid peninsular regions (280), whereas *D. repens* has been reported only along the Mediterranean coast, with a markedly elevated prevalence in some locations (37.1%) (73). In France, *D. repens* was reported to be more common than *D. immitis* (89) and is present in 19 of 62 districts, with an estimated mean national prevalence of 1.3% (418). The geographic distribution of *D. immitis* reaches a latitude of 50°N (124). In Italy, the prevalence of *D. immitis* exceeds 50% in the broad area of endemicity of the Po river valley, and more moderate rates are observed in the central areas (147); in contrast, *D. repens* is present throughout the country (420), with a prevalence rate of 30% in the Po river valley (418). The prevalence rates of *D. immitis* and *D. repens* in Greece are 10% and 30%, respectively (333), and historical data revealed the presence of *D. repens* in the Ukraine by Petropaulovsky in 1904 and in Russia by Gurvich in 1929, as reviewed by Artamonova (13).

Epidemiological studies and recent clinical reports on the expansion and emergence of dirofilariasis described a significant prevalence of autochthonous infections of dogs with *D. immitis* and/or *D. repens* in central and northern European countries where canine dirofilariasis was previously not reported or where cases were only sporadically found (see Fig. S1 in the supplemental material) (108, 123, 126, 149, 312, 319, 332, 349, 398, 409, 410, 455, 459). Cases of dirofilariasis were first reported in southern Switzerland in 1995 (109). A subsequent epidemiological study found *D. immitis* and *D. repens* in 6% of the dogs surveyed in this country, which was regarded as being free of dirofilariasis until those reports were published (339), with the most likely source of the introduction of the parasite being the border region near the Po river valley (Italy) (147). In the area surrounding Rostov-on-Don (southern Russia), the epidemiological profile has substantially changed over the last decade. In 1997, a prevalence of 30% was reported for *D. repens* (14), whereas in 2011, 20.25% of dogs were infected with *Dirofilaria* spp., 44.7% of which were infected with *D. repens*, 30.3% of which were infected with *D. immitis*, and

25% of which were coinfecting with both species (197). Distribution patterns are also changing in the countries of southern Europe where *Dirofilaria* spp. are traditionally endemic. In Spain, a significant prevalence of *D. immitis* has been reported for two northern provinces for which historical data on dirofilariasis are not available (294, 392). In Italy, epidemiological data from several decades (1960 to 2000) reveal both a geographical expansion and an increasing prevalence of *D. immitis* in northern regions where it is hyperendemic (147), the establishment of new foci of *D. immitis* and *D. repens* in the central regions (426), and an increase in their prevalence in regions that previously experienced only sporadic cases (137, 149, 376).

**Africa.** The distribution of canine dirofilariasis in Africa is not well known due to the paucity of epidemiological studies, the lack of methodological details regarding the assays employed for those studies, and the large variety of filaria species endemic to this continent. Genchi et al. (147) reported the presence of *D. immitis* in dogs in Morocco, Tunisia, Egypt, Tanzania, Kenya, Mozambique, Malawi, Senegal, Angola, Gabon, and Nigeria and in both dogs and cats in Sierra Leone (Fig. 5). In South Africa, autochthonous *D. repens* infections have been reported, whereas *D. immitis* infections seem to have been imported (147).

**Asia and Australia.** Historical data reviewed previously by Artamonova et al. (14) indicate the presence of *D. immitis* in various countries of central Asia. *D. immitis* has also been found in canine populations in Iran, with variable prevalence rates in different regions of the country (24). In India, *D. immitis* is distributed primarily across the northeastern region, and the presence of both *D. immitis* and *D. repens* in the province of Delhi has been confirmed (271, 334). To date, the highest prevalence rates of *D. repens* are found in Iran (61%) and in Sri Lanka (60%) (418). In Malaysia, the prevalence of *D. immitis* has reached 70% in some areas (reviewed in reference 147). In Japan, studies reported in the last decades showed high prevalence rates for *D. immitis* (46.8% and 59%) (143, 310). Elevated prevalence rates have also been found for South Korea and Taiwan (227, 403, 453). In Australia, *D. immitis* is endemic along the coast and in the eastern provinces, with prevalence rates similar to those found for the southwestern coast of the United States (46) (Fig. 5). Both *D. immitis* and *D. repens* have been reported in New Zealand but do not seem to be established in this country, with *D. immitis* periodically being diagnosed in dogs imported from Australia (264).

### Feline Dirofilariasis

An exhaustive review of the status of global feline dirofilariasis based on clinical reports and epidemiological surveys was presented previously by Newcombe and Ryan (309). According to that study, feline *D. immitis* infections tend to be detected in the same areas as canine infections albeit with prevalence rates that are the 5 to 20% of those found for dogs. In the United States, feline dirofilariasis has been reported in 29 states, with prevalence rates from 3% to 19%, with the highest rates in the areas with the highest levels of endemicity in dogs, and has been found in both feral and domestic cats. Reports have also described the presence of feline dirofilariasis in Canada, Brazil, and Venezuela (309).

In Europe, feline dirofilariasis has been found in northern Italy, where Kramer and Genchi (212) reported a prevalence rate of 7 to 27% in the hyperendemic region of the Po river valley. In the Canary Islands, two seroepidemiological studies showed an increase in feline dirofilariasis prevalences from 18.3% to 33% be-



tween 2004 and 2011 (281, 289). In Japan, a prevalence rate of 2% has been shown for the Kanto region (143), and a prevalence rate of 3 to 5.2% has been found for domestic cats on the islands of Honshu and Kyushu (362), whereas in South Korea, 2.6% of feral cats harbored parasites (240). In Australia, adult worms were found in 2 of 101 analyzed cats from the suburbs of Sydney, and 3 others tested positive for antigens (199). Various studies have shown that feline dirofilariasis is present in other countries, such as Sierra Leone, Armenia, China, the Philippines, Malaysia, Tahiti, and Papua New Guinea (309).

### Dirofilariasis in Wild Carnivores

Microfilaremic infections of various species of wild carnivores by *D. immitis* in many U.S. states have well documented (101, 141, 308, 318). Most of those studies were performed on carnivores with peridomestic habits, such as coyotes (*Canis latrans*), which may constitute an excellent sentinel for the spread of *D. immitis* (308, 367, 452), and foxes (*Vulpes vulpes*) (105, 452), with additional sporadic reports of infections in other wildlife carnivores (261). Highly variable prevalence rates have been reported, ranging from 16% in Illinois (308) and 43% in Florida (139) to 71 to 100% in coyotes, red wolves, and their hybrids in Texas, where *D. immitis* is considered an important cause of morbidity and mortality for these species (101). The expansion of dirofilariasis in coyote populations has been studied in California, where it has been demonstrated that the Sierra Nevada focus is stable, because the prevalence in this area did not change substantially from the period of 1975 to 1985 to the period of 2000 to 2002 (from 35% to 42%). However, in two other foci, the Northern California Coastal Range and south of the San Francisco Bay, the prevalence increased 4-fold throughout the same period and reached levels similar to those reported for the Sierra Nevada region, suggesting a spread of the parasite from this region toward the Pacific coast (366). *D. immitis* has also been identified in wild canines of South America. In a study of disease incidence among protected and released wolves (*Chrysocyon brachyurus*) on a natural reserve in Bolivia, most of the captive animals were infected with *D. immitis* (67). One epidemiological study revealed that 6.4% of red foxes in the areas surrounding Sydney (Australia) were infected with *D. immitis* (257).

The presence of *D. immitis* in foxes and jackals (*Canis aureus*) in numerous European countries has been described. In Spain, one infection of a wolf and sporadic infections of foxes have been reported for different areas of the country (154, 250, 383). In a study conducted on different ecosystems, the highest prevalence rate was found for agricultural areas (32%), whereas semiarid regions showed much lower rates (1.7%), and no infections were found in mountain foxes (161). Foxes infected with both *D. immitis* and *D. repens* in Italy (247, 254) and foxes and jackals infected with *D. immitis* in Bulgaria (reviewed in reference 261) have been found. Although infections in wild carnivores have been considered accidental and irrelevant for pets (318), interactions among wildlife reservoirs, pets, and humans may be intense in agricultural or suburban areas, where peridomestic interactions could occur (161, 257). Thus, the true impact of wild infections on the transmission dynamics of dirofilariasis should be carefully assessed.

The presence of *D. immitis* in many wild cats (ocelots, jaguars, lions, tigers, cougars, and leopards) and in black bears, both in the wild and in captivity in zoos, has also been reported. Nevertheless,

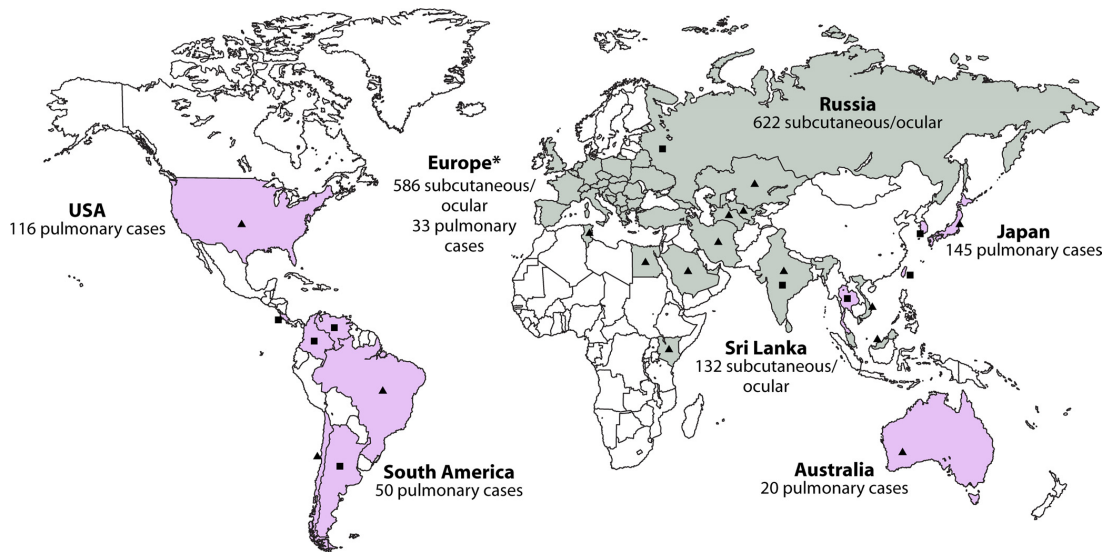
the lack of microfilariae in the reported infections indicates that these hosts play no significant role in the transmission of this disease (261).

### Dirofilariasis in Human Hosts

Wherever canine dirofilariasis exists, there is a risk of human infection. Epidemiological studies of human dirofilariasis have followed two different approaches: retrospective reviews of previously published cases and seroepidemiological analyses. Each of these approaches provides information on different yet complementary aspects of human infections. The following data are based on previously reported retrospective reviews (103, 148, 197, 226, 302, 328, 391) and on bibliographic searches for recently reported cases. The worldwide distribution of human dirofilariasis, as it is currently known (Fig. 6), does not coincide exactly with that of canine dirofilariasis, mainly due to the lack of data. There are countries with reported human cases in which data regarding canine infection are unavailable and vice versa; e.g., cases of human subcutaneous dirofilariasis caused by *D. repens* have been found in countries where only canine *D. immitis* dirofilariasis has been described. Approximately 1,782 human dirofilariasis cases have been reported, 372 of which were pulmonary and 1,410 of which were subcutaneous/ocular cases.

In the Americas, *D. immitis* pulmonary dirofilariasis predominates. In the United States, 116 cases have been reported, originating primarily in the southeast regions, where canine prevalence rates are highest (47, 226, 285, 299, 300, 400, 411, 423, 448). In South America, around 50 cases have been found, mainly in southwestern Brazil (273, 358, 442) and sporadically in Costa Rica, Argentina, Venezuela, and Colombia (39, 65, 370, 442). Regarding reported cases of subcutaneous/ocular dirofilariasis in North America, these have been frequently attributed to *Dirofilaria tenuis* and *Dirofilaria ursi* or *D. ursi*-like species (92, 113, 173, 179, 200, 314, 315, 450, 451) and to *Dirofilaria* spp. and *D. immitis* in a single case (423). In South America, two cases were recently reported: one subcutaneous infection in Chile which was attributed to *Dirofilaria* spp. (the first report from Chile) (337) and one ocular infection in Brazil caused by a species that was morphologically and phylogenetically similar, but not identical, to *D. immitis* (317).

In the Old World, where *D. immitis* and *D. repens* cocirculate in populations of animal reservoirs, subcutaneous/ocular dirofilariasis cases involving *D. repens* ( $n = 1,370$ ) largely predominate over pulmonary dirofilariasis cases (35), even in areas where *D. immitis* is highly endemic (391). On the contrary, in Japan, most human cases (152) have been attributed to *D. immitis* (226, 275, 368). Numerous human cases have also been reported for the European Union (586 subcutaneous/ocular and 33 pulmonary cases), Russia (622 subcutaneous/ocular and 3 pulmonary cases), and Sri Lanka (more than 132 subcutaneous/ocular cases). Subcutaneous/ocular infections caused by *Dirofilaria* species other than *D. repens* are quite rare in the Old World (45, 315). Until 1999, most reported cases originated from Mediterranean nations where *Dirofilaria* spp. are traditionally endemic (Italy, France, Greece, and Spain) (302, 328), with sporadic reports of small outbreaks of subcutaneous/ocular infections in Germany, the Netherlands, the United Kingdom, and Norway (302). Over the following decade, more cases were reported in Mediterranean countries (5, 9, 106, 128, 138, 155, 160, 177, 183, 201, 274, 323, 325, 326, 329, 331, 343, 353, 357, 379, 387); at the same time, a series of cases was described



**FIG 6** Current distribution of human dirofilariasis. Purple, countries in which *D. immitis* cases predominate; gray, countries in which *D. repens* cases predominate; ■, sporadic pulmonary cases; ▲, sporadic subcutaneous/ocular cases. The asterisk indicates that data from European countries except for the former Soviet Union were included.

in Turkey, Serbia, Croatia, Hungary, and Austria, and sporadic cases occurred in seven other countries (Table 2) (19, 20, 25, 44, 54, 127, 148, 209, 217, 244, 341, 412, 461). In this decade (2000 to 2010), subcutaneous/ocular dirofilariasis expanded from southern to central and northern Europe (149, 391, 393). By reviewing

**TABLE 2** Epidemiological data on human dirofilariasis, with special reference to Europe

Country	No. of cases					
	Pulmonary dirofilariasis			Subcutaneous dirofilariasis		
	Until 1999	2000–2011	Total	Until 1999	2000–2011	Total
Spain	5	3	8	6	2	8
France	2	2	4	24	63	87
Italy	3	10	13	135	188	323
Greece		3	3	10	25	35
Hungary			0		31	31
Croatia			0		10	10
Serbia			0	3	19	22
Germany	2	1	3	6	3	9
Turkey			0	1	21	22
Russia		2	2	61	561	622
Austria			0		>16	>16
Ukraine			0	23	1	24
Others <sup>a</sup>						170

<sup>a</sup> Countries with subcutaneous/ocular sporadic or imported cases due to *D. repens* include Bulgaria, Dubai, Georgia, Kazakhstan, Kenya, Iran, Israel, India, Japan, Malaysia, Poland, Romania, Slovakia, Slovenia, Sri Lanka (>132 cases), Tunisia (10 cases), Turkmenistan, Vietnam, Uzbekistan, Egypt, Saudi Arabia, Kuwait, Norway, Belgium, Australia, Brazil, Chile, and the United States. Countries with pulmonary sporadic dirofilariasis cases attributed to *D. immitis* include India, South Korea, Thailand, Taiwan, Costa Rica, Argentina, Venezuela, and Colombia.

data for the whole territory of Russia between 1915 and 2008, we have found that researchers reported 570 subcutaneous/ocular dirofilariasis cases (103). Fifty-two additional cases have been reported for various regions throughout Russia after that date (13, 35, 110, 408), along with three more cases of pulmonary filariasis (132, 197), with the nation’s southwestern regions accounting for the majority of cases (197). In Australia, 18 pulmonary cases had been reported until 1999 (66, 176, 283, 284), and only two additional cases, one pulmonary (303) and one subcutaneous (404), have been reported since then. One pulmonary case (probably imported from Fiji) has been reported for New Zealand (189).

Retrospective reviews of reported cases offer only a partial view of human dirofilariasis; i.e., they portray only “the tip of the iceberg” (391), and regions of endemicity showing vectors with zoonophilic habits probably have higher frequencies of human infections than reported in the literature. This underreporting could be related to the fact that symptoms in dirofilariasis patients, especially in pulmonary infections, may go unnoticed or be misdiagnosed.

Antibody detection in the blood allows the evaluation of the risk of dirofilariasis infection in a defined geographical region. Seroprevalence studies of residents of areas of endemicity reported high rates of infection similar to those of canine reservoirs of the same areas: 21% and 28% for *D. immitis* in the western Iberian Peninsula and Tenerife (Canary Islands), respectively (129, 344, 395); 10 to 30% in different isoclimatic regions of Grand Canary Island (282); 32.3% for *D. immitis* in northern Italy and 19.5% for *D. repens* in Sicily (345); and 10.4% for *Dirofilaria* spp. in southern Russia (197). In the United States, some epidemiological studies have registered seroprevalences of 13.8% and 16.3% for anti-*D. immitis* IgG and IgE, respectively, in children of Hawaii (112); 27% (IgG) and 5% (IgE) in inhabitants of Maryland

TABLE 3 Species of mosquitoes whose vector capacities for *D. immitis* have been demonstrated

Species	Location (reference[s])
<i>Aedes aegypti</i>	French Polynesia (365)
<i>Aedes albopictus</i>	Argentina (442)
	Italy (74, 77)
	United States (95, 235)
<i>Aedes caspius</i>	Taiwan (221)
	Italy (76)
<i>Aedes crucians</i>	United States (235)
<i>Aedes notoscriptus</i>	Australia (366)
<i>Aedes ochlerotatus</i>	America (243)
<i>Aedes punctipennis</i>	United States (235)
<i>Aedes punctor</i>	Italy (75)
<i>Aedes scapularis</i>	Brazil (219)
<i>Aedes sierrensis</i>	Utah (United States) (380)
<i>Aedes taeniorhynchus</i>	Brazil (4, 59)
	Yucatan (Mexico) (253)
<i>Aedes triseriatus</i>	Missouri (United States) (107)
<i>Aedes vexans</i>	Turkey (460)
<i>Anopheles maculipennis</i>	Italy (76)
<i>Culiseta incidens</i>	California (United States) (424)
<i>Culex annulirostris</i>	Australia (366)
	Italy (74, 76)
<i>Culex pipiens</i>	Argentina (442)
	Spain (286)
	Turkey (460)
<i>Culex quinquefasciatus</i>	Brazil (4)
<i>Culex theileri</i>	Madeira (Portugal) (371)
	Iran (23)

(230); and 5.2% in Illinois (378). In addition, serological techniques have allowed the identification of human dirofilariasis in areas and contexts in which it otherwise would not be detected due to their isolation and lack of diagnostic resources, such as in an indigenous Tikuna community in the Amazon in which dogs carry *D. immitis* (445). These data suggest that the risk of human infection by either *D. immitis* or *D. repens* is much higher than retrospective reviews of previously reported cases would indicate.

#### **Dirofilaria spp. in Vector Species**

Culicid mosquitoes are highly diverse. More than 3,000 species have adapted to a range of habitats that extend from coastal areas to mountain ranges. Few studies have aimed at determining how many of those species are involved in the transmission of *D. immitis* and *D. repens*. Reviews by Cancrini and Kramer (75) and Cancrini and Gabrielli (74) reported that multiple species of *Aedes*, *Culex*, and *Anopheles* allow the development of both of the above-mentioned *Dirofilaria* species. Further studies with animal/human bait traps performed in the United States, Italy, and Brazil have determined which vector species are preferentially attracted by different hosts of *Dirofilaria* spp. Specific identification by the localization of enzymatic activity in recovered larvae or PCR-based filarial DNA amplification (130) has demonstrated vector activity for *D. repens* by *Anopheles maculipennis* (142), *Aedes aegypti* (94), *Mansonia uniformis* (41), *Mansonia annulifera* and *Armigeres obturbans* (171), and *Aedes albopictus* (77). The species that have been implicated in *D. immitis* transmission (all of which belong to the genera *Culex*, *Aedes*, *Anopheles*, and *Culiseta*) are listed in Table 3. Epidemiological studies of vectors from around the world indicate that the prevalence of *Dirofilaria* is lower in

mosquitoes than in vertebrate hosts: 1.06 to 1.77% in *Aedes polyneisensis* in American Polynesia (85), 1.04% in *Aedes vexans* in Turkey (51), 6.2% in *Aedes taeniorhynchus* in the Yucatan Peninsula (Mexico) (253), 10% in *Culex theileri* in Iran (23), 2.3% in *Ae. albopictus*, 1.38% in *Anopheles crucians* and 0.85% in *An. punctipennis* in Georgia (235), and 3.8% in *Culex pipiens* in Turkey (460).

#### **Relevant Research Approaches to Climatic Change and Other Factors That Influence Transmission and Distribution Dynamics of *Dirofilaria* spp.**

The transmission of *Dirofilaria* spp. in a given region depends on the presence of a minimum number of dogs infected with adult worms producing microfilariae and on the presence of one or more mosquito species capable of transmitting the parasite. Therefore, dirofilariasis transmission is influenced by two factors that affect each of the two components of the worms' life cycle: (i) human behavior with respect to pets and (ii) climatic factors that allow for the presence of competent vector populations and *Dirofilaria* sp. larval development in these vectors.

The presence of infected dogs depends largely on pet management approaches, including the correct diagnosis and application of chemoprophylaxis, trips to and from regions of endemicity, and changes of residence. Some of the facts reported in the literature demonstrate this influence. In Canada, the emergence of infection foci in British Columbia has been documented and was attributed to hunting dogs imported from Texas (462). In the United States, after Hurricane Katrina, many dogs and cats were relocated to other regions of the United States and Canada. Among the relocated animals, 48.8% of dogs and 3.9% of cats were positive for heartworm, thus contributing to the geographical dissemination of dirofilariasis (229). In Grand Canary Island, the prevalence of *D. immitis* increased from 28% to 58.9% between 1988 and 1998 (279, 360), followed by a decrease to 19.2% in 2011 (281), coinciding with the widespread use of chemoprophylaxis. However, the prevalence remains at 40% in Podenco Canario hounds (a native breed of hunting and guard dog), which are kept in poor conditions and exposed to mosquitoes without chemoprophylaxis (279, 282). Compared with *D. immitis*, the rate at which *D. repens* is being introduced into central and eastern European nations is attributed to, among other factors, dogs acquiring subcutaneous dirofilariasis in southern regions of endemicity that go undiagnosed due to the asymptomatic course of the disease, with the dogs thus remaining untreated and becoming reservoirs that spread the infection beyond the region of endemicity in which they were originally infected when moved (148).

Mosquito colonization, development, and activity are regulated by climate, primarily temperature and humidity, and temperature also influences *Dirofilaria* sp. L3 development inside mosquitoes. It has been experimentally demonstrated that the development of infectious L3 larvae requires 8 to 10 days at 28°C to 30°C, 11 to 12 days at 24°C, and 16 to 20 days at 22°C. Below 14°C, development arrests, although it can be restarted when the temperature increases above this threshold (74). Consequently, the climate and its changes determine the presence and dynamics of transmission of dirofilariasis in temperate regions (149). A good example of the impact of climate on the distribution and prevalence of dirofilariasis is illustrated by Grand Canary Island, where the semitropical climate changes with altitude. Four isoclimatic zones have been defined, with marked temperature and humidity differences

among them, and the prevalences of *D. immitis* among the canine populations of each zone are significantly different: 25.47% and 30.4% in the stepparic and mild climate zones, respectively; 13.57% in the dry desert climate zone; and 10% in the temperate cold climate zone (282).

There is currently a scientific consensus regarding the phenomenon of climate change due to human activities (427). It is estimated that a total increase of 1.1°C to 6.4°C in temperature all over the world will be effected by the end of this century (186). Global warming affects host-parasite systems by influencing the amplification and emergence of parasite populations, inducing changes in the development and survival rates of both parasites and vectors, and altering seasonal transmission dynamics (68). With respect to dirofilariasis, climate change is lengthening annual periods of mosquito activity, shortening larval development periods, and increasing transmission in many geographical regions.

Human activities such as the urbanization of wilderness areas at city peripheries and the construction of irrigation and artificial aqueduct systems also contribute to the spread of dirofilariasis by creating microhabitats that favor vector population growth in regions where such habitats do not naturally exist. The combination of those changes with climate change is driving mosquitoes into large areas of temperate regions and contributing to changes in the global distribution of dirofilariasis. One example is the Asian tiger mosquito, *Ae. albopictus*, which was introduced into the Americas and Europe by the commercial tire trade through tires harboring mosquito larvae and which is spreading rapidly in newly colonized areas (3, 165, 352) and becoming established in regions on both continents where dirofilariasis is endemic. *Ae. albopictus* feeds on a broad range of mammalian species, including humans, and has adapted its development to environmental conditions in temperate areas (120, 361). *Ae. albopictus* has been shown to be a natural vector for both *D. immitis* and *D. repens* in Italy and for *D. immitis* in Taiwan and the United States (79, 221, 235). It is likely that *Ae. albopictus* vector activity for *Dirofilaria* will continue to spread; indeed, its introduction into other areas where dirofilariasis is endemic, such as the Mediterranean coasts of France and Spain, has already been observed (359, 375). The stable presence of this vector increases the risk of infection for animals and humans because its diurnal activity adds to the nocturnal activity of indigenous mosquito species (74).

The variety and abundance of vector species, their feeding predilections and activity patterns, the numbers of parasitic mosquitoes that survive infection, and the number of larvae that complete development to L3 are all factors that determine the efficiency of dirofilariasis transmission in a given area (75). For example, *C. pipiens*, which has been found to be infected by *Dirofilaria* spp., is highly abundant in various regions of endemicity in Europe and Asia, and its populations remain active throughout the summer; consequently, *C. pipiens* is considered a potential primary vector for dirofilariasis wherever it resides (10, 30, 286), although other species in the same areas can contribute to transmission. Therefore, the influences of all of these factors must be evaluated together. When mosquitoes are abundant, a reduction of the number of reservoirs is not sufficient to reduce the risk of infection. Otherwise, the spread of the disease across previously infection-free regions permits parasite introduction into naïve canine populations, which are less resistant than those residing in regions of endemicity.



FIG 7 Approximate length of the transmission periods of *Dirofilaria* spp. per year in Europe (calculated with data from references 142 and 149).

**Novel tools for the study and prediction of changes in the distribution of dirofilariasis.** Because climate plays a fundamental role in the epidemiology of vector-borne diseases, including dirofilariasis, and considering the trend of increasing environmental temperatures, it is necessary to apply surveillance protocols to detect those changes and apply adequate preventive measures when needed (413). Geographic Information Systems (GIS) comprises tools for gathering, analyzing, and presenting spatial data, and remote sensing (RS) is a technique that allows the study of objects without direct contact via image capture. These systems have two important applications to health research: (i) disease mapping and (ii) the development of predictive models for disease transmission dynamics (356). Both of these applications are important as a basis for decision making regarding the control of parasitic diseases.

As mentioned above, L3 development progresses exclusively at temperatures above 14°C until the accumulation of enough “degree-days” allows the maturation of infective L3 larvae. Degree-days are calculated in terms of heartworm development units (HDUs) (401). Complete L3 development requires 130 HDUs within the mosquito life span, which is estimated to be 30 days, and development will be completed independent of temperature fluctuations below the 14°C threshold during this period (245). Based on these calculations and on temperature and rainfall records from meteorological stations, together with information on localization and other temporal and geographical factors in the regions studied, a series of surveys using GIS and RS has been performed to create predictive models for dirofilariasis. Studies in Europe have allowed the determination of the number of annual *D. immitis* generations and the length of periods of high infection risk throughout several regions of the continent (Fig. 7) (148, 149, 150). These studies have shown that summer temperatures are enough to foster the extrinsic incubation of *Dirofilaria* even in colder climates, if dirofilariasis were to be introduced to the reservoirs of these areas. Therefore, if the current trend of rising



temperatures continues, it is estimated that within a few years, dirofilariasis could be introduced into areas in central and northern Europe where it is not currently endemic. These predictions seem to have been confirmed by epidemiological studies over the past decade that demonstrate both the emergence and the reemergence of dirofilariasis in humans and in animal reservoirs, as mentioned above. Related calculations regarding L3 development in vectors and the temperature requirements for it have been used by other authors to study the epidemiology of dirofilariasis in very specific areas. For example, Medlock et al. (270) demonstrated that in the United Kingdom, the temperatures during two summer seasons between 1995 and 2000 were warm enough to allow the complete development of *Dirofilaria* larvae in the southern regions, and in five of those summers, such conditions allowed L3 transmission in the areas surrounding London. Previously, Medlock et al. (269) studied the likelihood of the introduction of *Ae. albopictus* into England and concluded that this species could adapt to conditions in the south of the island with potential activity in 4 to 5 months per year, thereby increasing the risk of dirofilariasis transmission. Studies by Vezzani et al. (441, 442) demonstrated that dirofilariasis in Argentina has a seasonal transmission pattern like that in Europe and established a potential transmission window of nearly 6 months for Buenos Aires.

#### CLINICAL FEATURES OF HUMAN AND ANIMAL DIROFILARIASIS

##### Heartworm Disease in Dogs, Cats, and Ferrets

**Dogs.** Canine cardiopulmonary dirofilariasis (heartworm disease) is a serious and potentially fatal disease caused primarily by adult *D. immitis* worms (144, 261, 440, 444) and their antigenic products, including *Wolbachia* symbiotic bacteria (216).

Heartworm disease usually develops a chronic progression, first showing vascular and pulmonary effects and eventually affecting the right chambers of the heart (Fig. 8) (432). The first lesions occur on the walls of the pulmonary arteries and are key to the subsequent development of pulmonary and cardiac pathology. After the arrival of worms in the pulmonary arteries, an enlargement of endothelial cells in the vascular tunica intima occurs, resulting in the narrowing of vessels (endarteritis). Additionally, intercellular spaces increase, cells begin to deform (their longitudinal axes are modified due to mechanical trauma) (440), and the elasticity of the arterial walls is altered (193). The damaged arterial surface facilitates the passage of albumin, plasma, and blood cells into the perivascular space, stimulating the proliferation of smooth muscle cells in the vascular tunica media, which migrate to the lumen and induce intravascular villus formation (proliferative pulmonary endarteritis) (Fig. 9A), to which endothelial-like cells and collagen fibers also contribute (72, 351). The severity of villus proliferation is related directly to the length of time of the infection, the parasite load, and the strength of the host's immune response. The arterial wall becomes rough and velvety in appearance, with a concomitant reduction of both the lumen and the compliance of the pulmonary arteries (440, 444). Pulmonary disease develops subsequent to these vascular changes. Fluids and proteins that diffuse across damaged vascular walls cause edema and inflammation of the lung parenchyma. Vascular lesions can lead to the rupture of lung vessels due to an abrupt increase in the cardiac load associated with exertion and due to hemoptysis or severe lung hemorrhage.

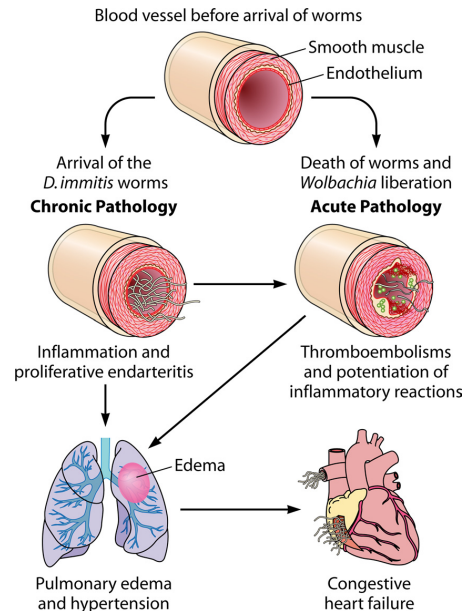


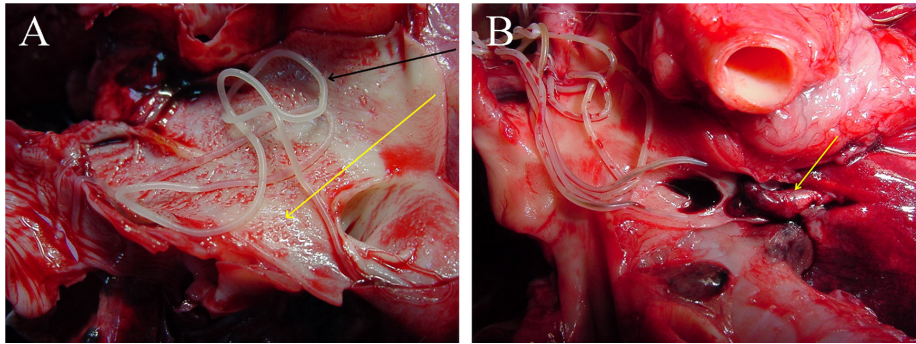
FIG 8 Progress of heartworm disease in dogs. The disease usually has a chronic progression. Initially, the damages affect the arteries, spreading later to the lung parenchyma and the right heart chambers. The simultaneous death of many worms contributes to the acute presentation.

Concurrent with the chronic progression of the disease, the death of worms, either spontaneously or by treatment with filaricides, causes acute adverse events characterized by thromboembolism (Fig. 9B) and severe inflammation, which threaten the survival of the affected animal.

The alteration of the arterial wall and the reduction in the luminal diameter of pulmonary arteries, which may be occluded by villi, thrombi, and/or the presence of worms, in conjunction with inflammatory mediators, can lead to pulmonary hypertension. This generates an overload of the right side of the heart, inducing cor pulmonale, a congestive right heart insufficiency with corresponding hypertrophy and dilation (Fig. 10A) that is worsened by changes in the tricuspid valve. In congestive right heart failure, there is generalized venous congestion as a consequence of increased systemic venous pressure.

*D. immitis* also causes severe renal dysfunction. Membranous glomerulonephritis has been described to be the result of alterations in the glomerular basal membrane in heartworm disease (2, 166, 203, 246). Glomerulonephritis is associated with the formation of immune complexes spurred by antigens from microfilariae, larvae, and adult worms, with the presence of microfilariae exacerbating the condition (321). Renal lesions can progress to severe nephrosis induced by proteinuria with renal insufficiency and azotemia.

A serious condition that is most frequently observed in small dogs is vena cava syndrome (VCS). This occurs via the displacement of a mass of worms from the pulmonary arteries to the right ventricle, where they interfere with the kinetics and function of the tricuspid valve, resulting in increased pressure in the right ventri-



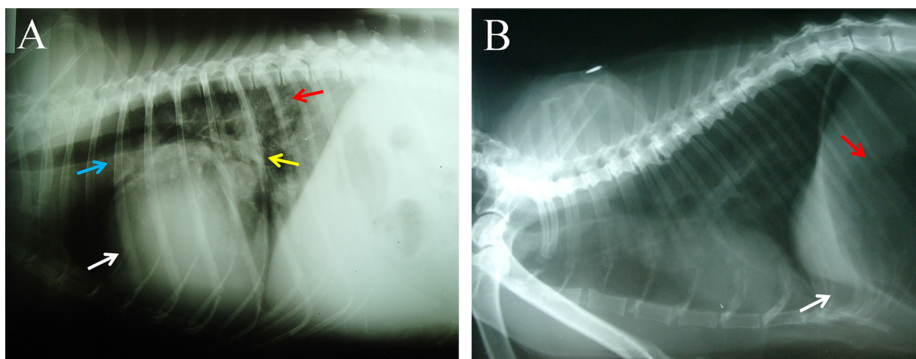
**FIG 9** Pathological alterations in pulmonary arteries associated with canine heartworm disease. (A) Surface of the vascular endothelium of a pulmonary artery from a dog with heartworm disease showing well-developed intravascular villi (yellow arrow). The black arrow indicates the presence of an adult worm. (B) Large thromboembolism (yellow arrow) in a pulmonary artery of a dog that died of cardiopulmonary dirofilariasis. (Courtesy of L. Venco, Clinica Veterinaria Città di Pavia, Pavia, Italy.)

cle and the obstruction of the valve lumen and circulating blood, thereby producing tricuspid insufficiency (144). These factors produce both volumetric and pressure overloads in the right atrium and the caudal vena cava, with a significant elevation in venous pressure and difficulty in return circulation. This situation often leads to the death of the animal due to hemolysis, hemoglobinuria, and disseminated intravascular coagulation (DIC).

Some dogs with occult dirofilariasis exhibit eosinophilic pneumonia that produces severe respiratory distress. This respiratory syndrome is caused by an eosinophilic inflammatory reaction to microfilarial antigens, which leads to alveolar dysfunction and impaired gas exchange, resulting in hypoxemia, hypoxia, and severe respiratory insufficiency.

Dirofilariae can produce lesions in other organs due to aberrant localizations, including the brain, liver, eyes, and peritoneal cavity. Many dogs do not manifest symptoms of these ailments for months or years, depending on parasite loads, individual reactivity, and animal exertion; arterial damage is greatest among animals that exercise vigorously. A frequent symptom in dogs is a

persistent, chronic, unproductive cough, which increases with exercise, followed by moderate or severe dyspnea and/or stress tachypnea. Respiratory insufficiency increases with disease progression. Infected animals can present with epistaxis, hemoptysis, or pulmonary hemorrhage, which, if severe, can result in hypovolemic shock. Infected dogs exhibit intolerance to exercise and weakness that may be accompanied by syncope associated with excitement or increased physical activity. When congestive heart failure develops, ascites, peripheral edema, hydrothorax, and hydropericardium appear. Venous ingurgitation (jugular, cutaneous, episcleral, or retinal) and visceral congestion also occur, leading to varied symptomatology. At the hepatic level, venous congestion causes hepatomegaly. This hepatopathy leads to liver failure, which may be accompanied by jaundice, increased levels of transferases, and coagulation disorders. Congestive splenomegaly also develops. Sudden death is rare but can occur as a consequence of cardiorespiratory insufficiency, cachexia, or severe thromboembolic phenomena. VCS produces orthopnea with severe respiratory insufficiency (hypoxemia/hypoxia), pansystolic



**FIG 10** X-ray images of canine and feline heartworm disease. (A) Lateral thoracic radiograph of an 8-year-old dog heavily parasitized by *D. immitis*. Cardiomegaly on the right heart (white arrow), a mixed pulmonary pattern (red arrow), enlarged pulmonary vessels (blue arrow), perihilar edema (yellow arrow), and large areas of pulmonary densification are noted. (B) Lateral thoracic radiograph of a cat with heartworm-associated respiratory disease. Note the lung air trapping as evidenced by the flattened and caudally displaced diaphragm (white arrow) and the gas-filled stomach, caused by aerophagia (red arrow).

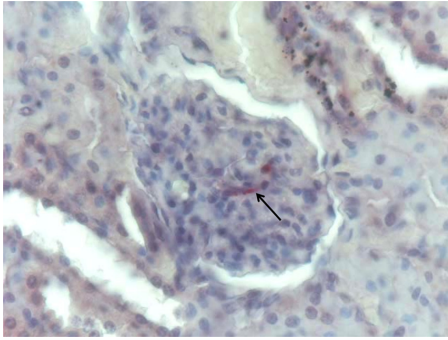


FIG 11 Anti-*Wolbachia* surface protein (WSP)-positive immunohistochemical reaction in a microfilaria from the kidney of a dog with heartworm disease (arrow).

heart murmur with tricuspid regurgitation, and hemoglobinuria by mechanical hemolysis due to turbulence caused by the interference of the accumulated worms with the blood flow (435). Extreme fatigue and complete exercise intolerance, as well as jugular pulse, DIC, decreased appetite, and, in the end, anorexia and death by cardiogenic shock, also occur. Finally, at the renal level, oliguria due to a decrease in filtration pressure and proteinuria following congestion-induced glomerular lesions may be present. Recently, IgG antibodies against *Wolbachia* were detected in the urine of dogs with heartworm disease, which is associated with the presence of microfilariae in renal capillaries (Fig. 11) and the release of *Wolbachia* when these microfilariae are destroyed (288).

**Cats.** Heartworm infection in cats is primarily pulmonary in nature. Infected cats can be asymptomatic carriers of the parasite or can present nonspecific clinical signs, most frequently respiratory or digestive in origin, such as chronic coughing, labored breathing, and vomiting. Some infected cats die suddenly without any premonitory signs (17, 239). When signs of infection are evident, they typically develop during the arrival of immature adult heartworms into the pulmonary vasculature and during the subsequent death of some of these worms, and signs may also develop during the death of adult heartworms. These signs are due to an acute vascular and parenchymal inflammatory response. Cats have specialized macrophages (pulmonary intravascular macrophages) in the capillary beds of the lung, and their activation is largely responsible for the exacerbated pulmonary reaction (119). The result is a nonfunctioning lung and an acute respiratory distress syndrome that is often misdiagnosed as asthma or allergic bronchitis but that is actually part of a syndrome known as heartworm-associated respiratory disease (Fig. 10B) (225). This term was coined to describe the lesions associated with the arrival and death of immature heartworms and can also be used for cats that develop the same symptoms due to the presence of adult heartworms (116). Heartworm-associated respiratory disease must be a part of the differential diagnosis when a cat presents with respiratory signs (119). In cats with either adult or immature heartworms, significant parenchymal and airway disease is manifested radiographically and histologically (17, 117). In addition, arterial and airway disease was demonstrated by Dillon et al. (117), who showed that histopathology scores for pulmonary arterioles, capillaries, bronchioles, and alveoli in *Dirofilaria*-positive cats are sig-

nificantly different from those of healthy cats (seronegative for *D. immitis*).

Histologically, the lung responds with eosinophil infiltrates in the parenchyma, pulmonary vasculature, and air spaces, causing pneumonitis. The pulmonary vessels may leak plasma and produce pulmonary edema, and due to the death of immature or adult heartworms, there is an increased level of activity of pulmonary intravascular macrophages. In conjunction with endotoxins from dead worms, cytokines, and possibly other inflammatory mediators, injury to type I alveolar cells occurs, with subsequent hyperplasia of type II alveolar cells (118). The result is diminished pulmonary function, hypoxemia, dyspnea, and cough; in some cats, this can lead to chronic respiratory disease. These findings provide evidence that pulmonary disease occurs in heartworm-infected cats, even when the infection does not progress to the adult worm stage, by inducing a strong vascular and parenchymal inflammatory response (53, 69, 114). This pronounced broncho-reactivity has been hypothesized to be due to the activity of pulmonary intravascular macrophages, a component of the reticuloendothelial system that cats, but not normal dogs, possess (118). The bronchial disease in cats with heartworm-associated respiratory disease is caused by a combination of epithelial disease, the proliferation of smooth muscle cells around the bronchioles, and an altered reactivity of bronchial smooth muscle cells to stimuli or an inhibited ability to respond to a bronchodilator; in fact, there is a loss of elasticity in the bronchioles, and the decreased lumen is more the result of epithelial infiltrates and smooth muscle cell proliferation than of bronchoconstriction (116). It has been hypothesized that the damaged cuticles of worms release large quantities of antigens and cause acute systemic shock. In an experimental model of acute systemic anaphylaxis in *D. immitis* antigen-sensitized cats, the intravenous inoculation of parasite antigens produced acute shock similar to that described above, which included dyspnea, hypoxia, and systemic hypotension (238) and for which a link between the severity of the response and the amount of inoculated antigen was demonstrated (237).

Hyperacute dirofilariasis involves severe respiratory symptoms (including respiratory insufficiency) and gastrointestinal, cardiovascular, and neurological signs (431). The most frequent symptoms include dyspnea, cough, tachypnea, vomiting that is not associated with food intake, and diarrhea. Aberrant L4 localization is more frequent in cats than in dogs, and larvae have been found in body cavities and the nervous system (16). In cases with an ectopic localization of worms, ataxia, syncope, vestibular alterations, and blindness may occur.

In nonfatal cases of acute infection, cats can transition to the chronic stage or become fully asymptomatic but later revert to the chronic form of the disease. Chronic dirofilariasis is generally associated with cases in which respiratory and/or gastrointestinal symptoms predominate, leading to severe organic degradation until the onset of cachexia (151, 431). There are no reports of congestive heart failure. In contrast, arterial wall lesions, although similar to those in dogs, are better endured by cats, which is attributed to the presence of fewer worms and a shorter duration of infection (115).

**Ferrets.** The ferret (*Mustela putorius*) has been an important hunting animal for centuries, and it has recently begun to be regarded as a pet. Ferrets are susceptible to dirofilariasis, and they have been used as an experimental host for heartworm studies (259). In ferrets, adult *D. immitis* worms are frequently located in



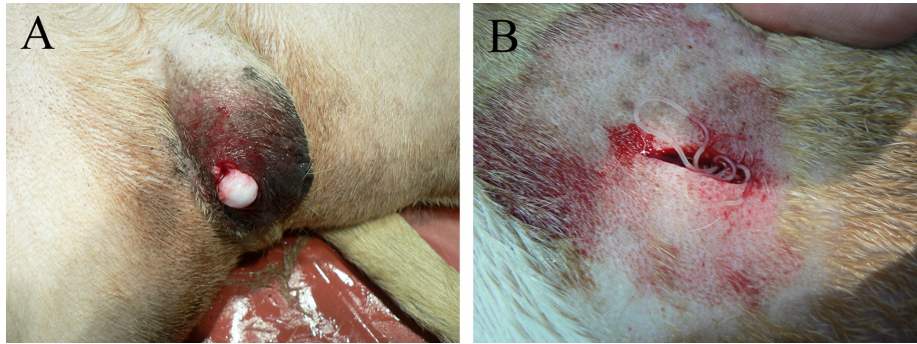


FIG 12 Canine subcutaneous dirofilariasis caused by *D. repens*. (A) Subcutaneous nodule in the scrotum of a male dog. (B) Adult worm in an open subcutaneous nodule. (Courtesy of Sergey Kartashov, Rostov, Russia.)

the cranial and cava veins as well as the pulmonary arteries and cardiac cavities (406). The presence of very few worms can cause serious symptoms and even the death of ferrets (198). Vena cava syndrome develops quite frequently, as indicated by a study by Supakorndej et al. (407) in which 1 out of 7 infected ferrets developed this syndrome. The symptoms are quite similar to those that occur in dogs, but they progress faster. These symptoms consist of lethargy, poor appetite, exercise intolerance, pleural effusion, cyanosis, and dyspnea. Hematological abnormalities such as anemia and monocytosis have also been shown. When sudden death occurs, this is caused by pulmonary embolisms (198).

#### Subcutaneous and Ocular Dirofilariasis in Animal Reservoirs

Subcutaneous dirofilariasis in dogs is commonly associated with the presence of adult *D. repens* worms in subcutaneous tissues (see Fig. S2 in the supplemental material) and/or subcutaneous nodules (Fig. 12) (164), although they can occupy other sites, including the ocular conjunctiva (178). The infection usually progresses asymptotically. Clinical manifestations have been classified into two clinical syndromes (377): multifocal nodular dermatitis, which is generally localized to the face, and prurigo papularis dermatitis. However, diverse dermatological signs that recur seasonally for years are common, such as pruritus in 100% of animals, erythema (79%), papules (62%), focal or multifocal alopecia (55%), hyperkeratosis (18%), crusting (14%), nodules (12%), acanthosis (5%), eczema (3%), pyoderma (3%), and edema (1%). Extradermic symptoms include conjunctivitis (46%), anorexia (35%), vomiting (26%), fever (25%), lethargy (20%), and lymphadenomegaly (10%) (417, 418). There are no experimental data regarding pathogenic mechanisms, although these alterations and lesions have been attributed to both mechanical and immunopathological processes (164).

Some reports described alterations to internal organs such as the spleen, liver, kidneys, lungs, heart, and brain that are associated with massive infections with adult worms and microfilariae (164). Cases involving intravitreal infection in dogs are unusual and may be caused by *D. repens* (172, 178) or, rarely, by *D. immitis* (102). The prognosis depends on the damage caused by the worms and the degree of success of surgical extraction (102).

#### Human Dirofilariasis

**Pulmonary dirofilariasis.** Human pulmonary dirofilariasis is characterized by the formation of pulmonary nodules (Fig. 13)

around immature adult worms that have recently molted from L4 larvae. When L4 larvae reach a small or medium branch of the pulmonary artery, they block its passage, causing embolism and localized inflammation (301). The most significant event with far-reaching consequences in human pulmonary dirofilariasis management is that the discovery of such nodules is frequently misdiagnosed as a malignant lesion (391). Gross histology reveals a central clot, which often traps a worm, surrounded by a yellowish or whitish fibrous wall 1 to 3 mm thick. In many cases, histopathology exposes worm structures at various stages of decomposition in the arterial lumen, surrounded by copious inflammatory infiltrates. In some cases, only a cellular reaction is observed because worms have already been destroyed by the time the nodule is found (391). Histological studies of lung nodules caused by *D. immitis* have shown that such cellular infiltration comprises eosinophils, lymphocytes, and plasma cells, accompanied by a histiocytic reaction and inflammatory changes in the tissues surrounding capillaries. These events are responsible for nodule for-

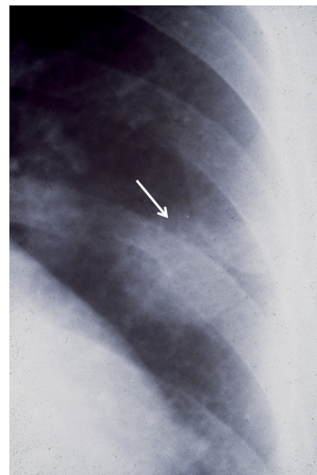


FIG 13 Human pulmonary dirofilariasis. Shown is a thoracic radiograph showing a pulmonary nodule attributed to *D. immitis* (arrow).



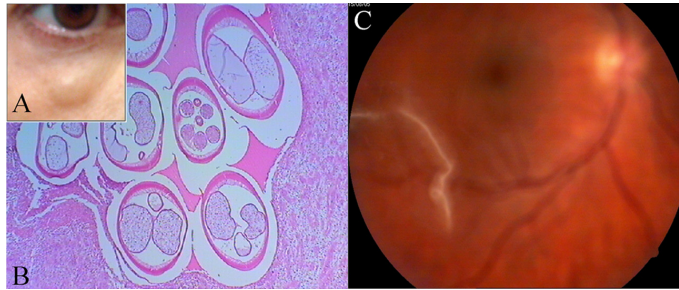


FIG 14 Human subcutaneous and ocular dirofilariasis. (A) External appearance of a subcutaneous nodule in the ocular region. (B) Histological section of a nodule showing sections of adult *D. repens* worms. (C) Intravitreal location of an adult *D. repens* worm in a human patient. (Panels A and B courtesy of Vladimir Kartashev, University of Rostov Na Donu, Rostov, Russia; panel C reprinted from reference 208 with permission.)

mation rather than infarction stemming from embolism formation. Necrotic regions with pulmonary artery disruption due to exiting worms are also frequently observed (12).

Although single nodules appear most frequently, multiple lesions have also been described, with a maximum of five occurring in the same individual (206). In general, radiological characteristics—spherical or ovoid nodules with well-defined borders and a homogeneous density—suggest a benign profile (301). Residual calcified lesions have also been described, which are consistent with the angiocentric lesions typical of dirofilariasis (97). In previous reports, times to nodule formation of 2, 3, and 8 months have been reported (191, 206, 304). To date, the longest-known residence of a single nodule is 13 years (42), during which the nodule underwent calcification, whereas a 2-year follow-up of a single nodule did not reveal any modifications to its radiological features (301). These lesions often disappear with time, suggesting that pulmonary dirofilariasis can present with transient lesions (98).

There is evidence that nodules tend to be most frequently found in the right lung although with no differences in lobar distribution. Nodules are commonly found in peripheral locations, usually in subpleural regions. Pulmonary dirofilariasis is detected at the highest frequencies in male adults with a mean age of 53 years, although infected patients range in age from 10 to 79 years (301). Only a small number of patients present with symptoms associated with pulmonary dirofilariasis. When these symptoms do arise, they are nonspecific and include coughing with pleural or nonpleural thoracic pain (at comparable frequencies), purulent or hemoptoic sputum with hemoptysis and dyspnea in the minority of cases, fever, and other nonspecific signs such as malaise and myalgia. Only one case was initially diagnosed as a pulmonary embolism. Nevertheless, this diagnosis is considered a possible cause of the symptoms in most symptomatic cases that show nodules on an X ray. Auscultation is almost always normal, with crepitation, stertor, and wheezing being the most frequent signs among abnormal profiles. When present, pleural effusion is of a low magnitude (301).

**Subcutaneous/ocular dirofilariasis.** Subcutaneous dirofilariasis, which is caused by adult and preadult *D. repens* worms in subcutaneous tissues, presents as a subcutaneous nodule (Fig. 14A and B) that grows gradually over a period of weeks or months. It has a firm, elastic consistency and is associated with erythema. Histology reveals four types of nodules, with diverse contents and

characteristics (327). Although the highest incidence of subcutaneous cases occurs in individuals aged 40 to 49 years, infections in patients of all ages have been described, most notably in Sri Lanka, where 33.6% of the reported infections have occurred in children under 10 years of age. In contrast to pulmonary dirofilariasis, women seem to be more susceptible to subcutaneous dirofilariasis than men (55.4% versus 44.6%).

The percentage of reported cases of ocular dirofilariasis has been increasing in recent years. Between 30% and 35% of *D. repens*-related infections occur in ocular regions (orbital zone, eyelids, and subconjunctival and intravitreal tissues) (Fig. 14C) (148, 328). Some of these cases have serious consequences, with symptoms including damaged vision, floaters, or loss of sight (148). Permanent complications, such as retinal detachment, glaucoma, opacity of the vitreous humor, crystalline lens, or other losses of visual acuity, will develop in 10% of patients (21). Additional risks and side effects are associated with the surgical extraction of worms from sensitive areas, such as the optic nerve (208). In cases with orbital localization, symptoms such as blepharodema, palpebral ptosis, and moderate ocular discomfort occur (404). Worms in the ocular conjunctiva can also cause inflammation in addition to hyperemic conjunctival tumefaction (364).

**Questionable paradigms related to human dirofilariasis.** The traditional picture of human dirofilariasis includes three concepts: (i) *D. immitis* is associated with pulmonary nodules, and *D. repens* is associated with subcutaneous nodules and ocular locations; (ii) human infections are caused only by immature worms; and (iii) human infections are sporadic and accidental. These paradigms are currently changing as a consequence of the data obtained in the last 10 years.

It is not infrequent to find worms of both species in anatomical locations distinct from those commonly associated with each species. *D. immitis* worms have been found in cranial, hepatic, intraocular, and mesenteric adipose tissues; testicular arteries; and conjunctival tissues (22, 423), and *D. repens* worms have been found in the lungs, scrotum, penis, spermatic cord, epididymis, and female mammary glands (148, 328). A questionable identification of the species causing dirofilariatic nodules (330) can be due to alterations in the parasite structures or to the decomposition of worms inside nodules. Under these conditions, the automatic attribution to a particular species due to the location of the nodule lesion and the use of diagnostic techniques with a reduced predictive value to confirm diagnosis, such as serological tech-

niques, seem inadequate to ascertain the causative *Dirofilaria* species in each particular case.

Regarding worm development in human patients, intact worms have been collected from subcutaneous nodules or ocular conjunctiva in some infections by *D. repens* (214, 315, 343, 364). Some of the collected *D. repens* worms were mature females carrying intrauterine embryos or microfilariae (214, 343). Moreover, at least three cases of subcutaneous human dirofilariasis showing circulating microfilariae have been reported (reviewed in reference 150). These data demonstrate that the full development and fertilization of *D. repens* worms in human hosts are feasible, contradicting the commonly accepted belief that *Dirofilaria* worms cannot fully develop in human patients. The reasons why these findings are restricted to *D. repens* must be carefully analyzed. It is likely that, as mentioned above, a subcutaneous/ocular localization facilitates a fast detection of the infection, being frequently referred to by the patients themselves, and as a result, many worms may still be intact upon diagnosis. In contrast, *D. immitis* pulmonary nodules are internal, and most are asymptomatic; thus, when they are diagnosed, they are more likely to be due to old infections, with the worms which caused the infection being unidentifiable due to the destruction and decomposition of parasite tissues. However, there are other factors that could influence the lack of reports of fully developed worms in patients with *D. immitis* to be considered. A recent analysis of *D. immitis* and *D. repens* proteomes and immunomes showed that *D. immitis* stimulates more vigorous antibody production against its energy metabolism and detoxification machinery than *D. repens*. If these specific antibodies can block the activity of parasitic enzymes, these data could account for the limited capacity for survival of infective *D. immitis* larvae over the infection time in human patients, due to worm metabolism blockage, and, consequently, could account for a lack of worm maturation in this host (158).

Currently, human dirofilariasis is considered an emerging disease in some areas (214) because of the dramatic increase in the number of reported human cases (mostly subcutaneous/ocular cases) in the last 10 years. This contradicts the concept that human dirofilariasis is accidental and infrequent. Geographic expansion and the increased prevalence among canine populations are likely to run in parallel with the increase in numbers of human cases. The frequently asymptomatic character of *D. repens* in dogs and, thus, the lack of its clinical diagnosis could contribute to its silent spread through canine reservoirs, increasing the risk of infection in humans residing in the same areas (148). Some other factors that are not related directly with the epidemiology of the disease could have contributed to the detection of an increased number of human cases in the last years. In this respect, more than 80% of all human cases have been identified in a small set of countries (see "Dirofilariasis in Human Hosts"), countries in which research groups traditionally working with human dirofilariasis are found. Not surprisingly, other countries with frequent canine dirofilariasis infections show sporadic or no reports of human infections. This shows that the medical community's knowledge of and vigilance for these parasites can decisively influence the successful detection of human cases, the numbers of which are likely to be underestimated in many areas of endemicity.

A second point of discussion is that the increase in the number of human cases is due mostly to subcutaneous/ocular reports, with few pulmonary cases being reported in Europe. This has been attributed to the presence of two different variants of *D. immitis* in

America and in Europe, with the Old World variant exhibiting no pathogenic role in humans. Furthermore, it has been pointed out that cases attributed to *D. immitis* in European patients may not be well characterized and instead represent *D. repens* infections (330), thereby rendering *D. repens* almost exclusively responsible for human infections in Europe. However, some data contradict this hypothesis. For example, it was demonstrated that *D. immitis* worms from diverse geographical origins, including Old World and New World locations, display genetic homogeneity (38, 182). In addition, some of the pulmonary cases from Spain that were determined to be caused by *D. immitis* based on radiological and serological evidence (96, 97, 98) were those of patients who had resided exclusively in regions where only *D. immitis* has been detected in canine populations. Nevertheless, the difference in the numbers of human cases of *D. repens* and *D. immitis* infections are unquestionable. This difference cannot be attributed solely to the easier detection and diagnosis of *D. repens* nodules and the missed, asymptomatic *D. immitis* pulmonary cases. In this respect, more accurate and complete epidemiological and clinical information should be obtained to ascertain the fact(s) to which this disparity can be attributed.

## Diagnosis

**Laboratory diagnosis.** Heartworm disease in dogs is diagnosed by the detection and specific identification of microfilariae and by using tests for the detection of circulating adult worm antigens, available only for *D. immitis*. Microfilariae in the blood are usually detected in concentrated blood specimens by microscopy using the Knott test or other tests to concentrate microfilariae (435). However, given the variety of canine filariae, the detection of microfilariae alone does not give an accurate diagnosis, because although filarial species can be identified by an evaluation of cephalic and caudal morphologies, these features are often difficult to differentiate. Species can also be defined by the histochemical staining of anatomical regions with phosphatase activity (84, 338) and by the amplification of microfilaria DNA by PCR (130, 131). *D. immitis* microfilariae harbor two phosphatase activity zones near the anal and excretory pores, whereas *D. repens* has only one near the anal pore. Other canine filariae exhibit different, distinguishable patterns of phosphatase activity. In PCR-based identification assays, primers based on the sequence of a *D. immitis* cuticle antigen-encoding gene with multiple tandem repeats (342) and also based on a second, highly repetitive sequence that constitutes 3% of the *D. repens* genome (86) have been used. Recently, a duplex real-time PCR able to detect *D. immitis* and differentiate it from *D. repens* in dogs and mosquitoes (223) and a multiplex PCR for the simultaneous detection of filarioids in dogs (224) have been described. Highly specific and sensitive enzyme-linked immunosorbent assays (ELISAs) or immunochromatography-based assays that detect circulating antigens of adult *D. immitis* females are commercially available for the diagnosis of cardiopulmonary dirofilariasis (389, 435). Serological tests allow the detection of amicrofilaremic infections, which are not detectable by using the techniques described above. In most cases, the use of a combination of the above-mentioned techniques allows the accurate detection of dirofilariasis. A positive microfilaria test followed by a positive antigen test conclusively confirms an infection with *D. immitis*. If microfilaria testing is positive but antigenic testing is negative, the infection is caused by a species other than *D. immitis*, which can be determined by histochemical phosphatase

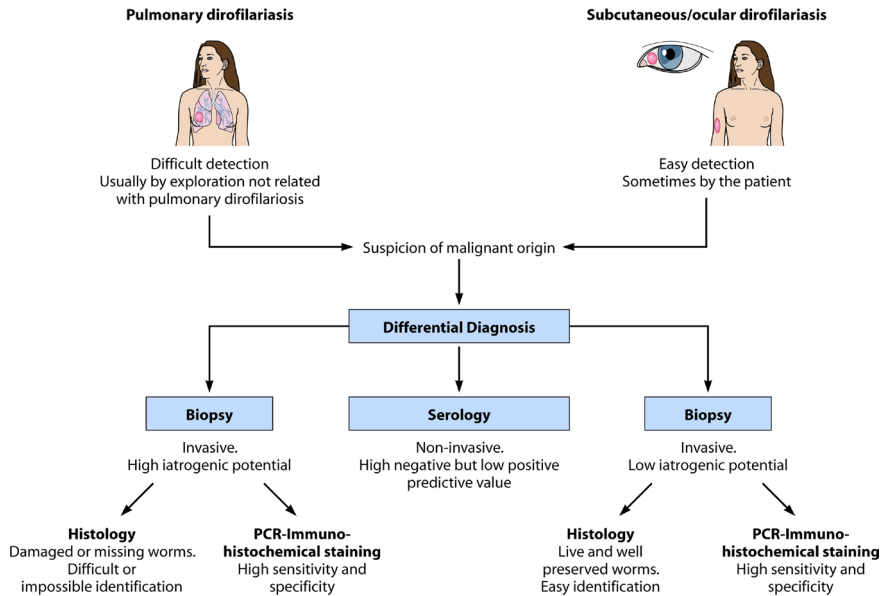


FIG 15 Management of human pulmonary and subcutaneous dirofilariasis.

testing or by PCR. This potential diagnostic alternative is also useful in cases where the infection is caused by very few female worms or when adult parasites have died by natural causes or by treatment with adulticides, which leaves only circulating microfilariae in the peripheral blood. Moreover, both the transplacental and transfusion-based transmissions of microfilariae lead to a positive microfilaria test with a negative antigen test. A positive antigen test without the detection of circulating microfilariae indicates an amicrofilaric infection with *D. immitis*.

Feline cardiopulmonary dirofilariasis is seldom diagnosed due to its asymptomatic nature. When diagnosed, the erratic progression of many infections and the lack of microfilariae in most cases (261) make the use of combined diagnostic techniques to diagnose the disease necessary. Antigen detection, which is regarded as the gold standard for detection in dogs due to its sensitivity and specificity, does not yield the same benefits for cats (121). In feline dirofilariasis, infections by few adult and preadult worms are common, resulting in low concentrations of antigens that can occasionally be derived exclusively from male worms and are therefore undetectable by antigen testing. Consequently, antigen detection usually produces false-negative results and underestimates the number of feline infections (40). However, it is important to highlight the diagnostic utility of antigen detection when the parasite load consists of a single female worm. Another diagnostic procedure is antibody testing, which indicates the presence of specific anti-*D. immitis* antibodies in infected hosts. The immune response in feline cardiopulmonary dirofilariasis is generally strong after approximately 2 m.p.i., thereby enabling the early detection of single-worm infections (346, 348). In contrast to antigen-detecting tests, circulating antibody tests can overestimate the number of infected animals by yielding false-positive results (40). Post-mortem examinations of animals that previously yielded negative

results by antibody tests have illustrated that false-negative results also occur. Moreover, cats with cardiopulmonary dirofilariasis without clinical symptoms may be more likely to produce negative antibody tests (307). Despite these limitations, serological testing is still a highly useful tool and can be employed in combination with other techniques to increase diagnostic precision in cats.

In ferrets, due to transient microfilaremia, the detection of microfilariae is not a valid method of detection in most cases. Tests for the detection of circulating antigens become positive 1 month earlier than tests for detection in dogs, probably due to their higher concentrations in the small blood volume of this host (198, 406).

Diagnosis is the key point in the management of human dirofilariasis. Human pulmonary and subcutaneous/ocular dirofilariasis pose different diagnostic challenges (Fig. 15). In the case of subcutaneous nodules or an ocular localization of worms, it is usually the patient who first discovers the infection and requests medical attention. In contrast, pulmonary nodules are located deep within the body and are asymptomatic in a high percentage of cases, and only a fraction of lung nodules are accidentally identified during chest X-ray procedures (Fig. 13), which are generally performed for reasons unrelated to dirofilariasis (98). However, in both cases, when nodules are detected, a malignancy is usually suspected, which makes human dirofilariasis an essential component of the differential detection of subcutaneous and pulmonary nodules (391).

During the diagnostic process, two main issues must be addressed: adequate sample collection and the accurate identification of the causal agent. In the absence of microfilariae in the blood, detection is usually performed via biopsies that determine the presence of worms in nodules. This is an invasive procedure with a high iatrogenic potential, especially for pulmonary dirofi-

lariasis. After the biopsy is performed, successful worm identification depends on several factors. One potential problem that is highly relevant to pulmonary dirofilariasis is the degree of worm decomposition in nodules, which makes their identification more difficult. An additional issue of concern is the similar morphologies of the cuticles of various species (262). In addition, some key features that are considered for worm species identification, including cuticular ridge size, ridge numbers, and ridge-to-ridge distance, are highly variable at different body areas of the same worm and even within a single transverse section, thereby rendering these criteria unreliable for diagnosis (315). According to the authors of that study, the localization of worms with smooth cuticles in subcutaneous tissues poses problems of identification because all species of the genus *Dirofilaria*, especially those described to infect humans, have cuticular ridges, except *D. immitis* and *D. lutrae*. In this respect, and because *D. lutrae* has not been found in humans to date and the initial stages of *D. immitis* develop within subcutaneous tissues, subcutaneous worms with smooth cuticles are usually identified as *D. immitis*.

Molecular and immunological techniques are currently available as an alternative or complement to morphology-based diagnostic techniques. For cases in which parasites exhibit altered morphologies due to the host reaction, parasite detection by PCR is an invaluable technique due to its high sensitivity and specificity. Positive reactions are obtained with minimal quantities of parasite DNA, even from samples conserved in different fixatives (131). Immunohistochemical staining to confirm the existence of *Wolbachia* or its molecules in nodules can also be helpful, because a positive reaction indicates the prior presence of *Dirofilaria* (390). Nevertheless, none of these techniques preclude surgical intervention to obtain biological material from nodules.

Despite the small number of worms that are required to cause infection, humans demonstrate a strong antibody response. Therefore, serology is used as a complementary technique to invasive methods. Different antigen complexes have been used to detect antibodies and diagnose human dirofilariasis (372, 395). ELISAs have been refined with *D. immitis* somatic antigen (DiSA) and excretory antigen (DiE/S) from adult worms, which can be relatively easily obtained. Nevertheless, those crude antigens can cross-react among different *Dirofilaria* species and with other parasitic helminths of humans, primarily *Toxocara canis* (agent of visceral larva migrans) (395). This problem of specificity can be resolved by using epitopes from polypeptide sequences found in antigen complexes with previously demonstrated specificity for *D. immitis* or *D. repens* (336, 397). The gene encoding Di35, a 35-kDa protein previously identified and characterized in its native state by Philipp and Davis (340), has already been cloned and produced as a recombinant antigen (405). When used in an ELISA, this recombinant protein demonstrated high sensitivity and specificity for human pulmonary dirofilariasis in studies of sera from patients with tropical filariasis. A group of molecules in the 22-kDa range (known as Di22) proved to be an excellent marker for pulmonary dirofilariasis (335) and has been used successfully in the diagnosis of clinical cases by Western blotting. In ELISAs, this protein exhibits 100% sensitivity and 90% specificity, with positive and negative predictive values of 75% and 100%, respectively (336). However, given the low pretest probability, positive results in serological studies need to be supplemented with other data, such as radiography, medical history, and region of residence, before any invasive diagnostic measures are initiated (302). With

respect to *D. repens*, many specific polypeptides have been identified in the range of 26 to 40 kDa. These molecules allow discrimination not only between subcutaneous dirofilariasis and other parasitic or nonparasitic diseases but also between clinical cases and infections without subcutaneous or ocular changes (397). Finally, there is a good correlation between PCR-based and serology-based results, as evidenced by a study of eight cases with subcutaneous nodules or ocular localization that were analyzed in parallel by using molecular techniques, ELISA, and Western blotting (78).

**Clinical diagnosis.** Visual assessments, such as chest X ray, echocardiography, and electrocardiography, provide insights into the clinical status of each individual patient. A chest X ray provides evidence of pulmonary artery enlargement (Fig. 10A), lung parenchymal changes, and right heart cardiomegaly in advanced stages of canine dirofilariasis (435, 443). This technique also allows the confirmation of the presence of pleural effusions (351), although it cannot be used to assess the parasite load (436). Using echocardiography, clinicians can visualize parasites, which are seen as two parallel hyperechoic lines in the main pulmonary artery, left and right interlobe branches, or right heart atrium and ventricle (26, 276). Doppler echocardiography enables the precise determination of the presence and severity of pulmonary hypertension. Echocardiography must be considered in cases in which clinical and radiographic features suggest a severe infection, because they provide data on the stage of the disease and the parasite load, which are key factors in the choice of appropriate therapy (435). For diseased dogs in terminal stages that exhibit severe enlargement of the right atrium, electrocardiography can reveal alterations in both the electrical axis and rhythm (deviations to the right side of the axis and atrial fibrillation) (435).

Several molecules released into the blood following cell damage in capillaries and myocardial tissue (311), inadequate perfusion (56), or lysis of thrombi (153) can serve as early markers for cardiovascular ailments and thereby assist in the treatment decision-making process (56, 298). Levels of troponins T and I, myoglobin, and D-dimer in dogs with heartworm disease have recently been studied (80). The preliminary results obtained indicate the feasibility of the use of troponin I and myoglobin as markers of cardiac damage and of D-dimer as a confirmatory tool for the diagnosis of pulmonary thromboembolism in dogs (80).

In feline cardiopulmonary dirofilariasis, a chest X ray allows a presumptive diagnosis and the evaluation of the severity of the infection based on characteristic changes of arteries and the lung parenchyma (Fig. 10B). Experimentally infected animals present with radiographic alterations 6 months after inoculation with infectious larvae (231). The presence of interstitial pulmonary patterning and radiodense zones in the lung parenchyma with enlargement, blunting, and tortuosity of one (usually the right) or both caudal pulmonary arteries must be regarded as suggestive of cardiopulmonary dirofilariasis in cats (18). Alterations in the cardiac silhouette are rare. It is also worth noting that radiographic profiles look normal in some cases despite the infection, and alterations in radiographic profiles may be transient (384), resulting in an erroneous diagnosis. Infections produced by a single parasite can also induce alterations that are not visible by radiography (437). Thus, it is essential that clinicians consider the advantages and limitations of each diagnostic technique and use all of the resources that they have at their disposal to reach an unequivocal diagnosis.

Echocardiography allows detection when filariae are located in anatomical regions accessible to ultrasound, such as the right heart chambers, main pulmonary artery, proximal tracts of the right and left pulmonary arteries, and the distal portion of the caudal vena cava (277). The dimensions of adult filariae are significant compared to those of the pulmonary arteries of cats, facilitating their identification (26, 71, 439). Finally, necropsy is recommended for suspected cases in which death preceded diagnosis or for those cases in which cardiopulmonary dirofilariasis cannot be excluded despite a negative diagnosis. In addition to the predictable anatomical locations, other regions should be examined to exclude the presence of filariae in ectopic sites, including the systemic arterial circuit, body cavity, and brain and spinal cord, in cases with neurological symptoms.

Radiographic changes in ferrets typically consist of cardiomegaly and severe pleural effusion (198, 406). Ultrasound can detect adult worms 5 months postinfection, 1 1/2 months earlier than in cats (384).

### Treatment and Prevention

Heartworm disease treatment in dogs is complex and frequently risky due to the side effects of the massive destruction of worms in the bloodstream. Therefore, it is necessary to choose an appropriate therapeutic strategy, and it may be best to withhold treatment in some cases.

Before treatment, the individual situation of each animal must be evaluated, considering factors such as parasite load, age, and size. Other concomitant risk factors are the severity of pulmonary disease and the restrictions on physical activity that the dog can realistically endure (122, 438). Based on all of these factors, the animal's risk of thromboembolic complications and the presence of VCS can be determined. Dogs in the low-risk group exhibit a low parasite load, the absence of lung parenchyma and/or capillary lesions, normal clinical symptomatology and chest X ray, low levels of circulating antigens or negative microfilaremic antigen tests, negative parasite tests by echocardiography, the absence of concomitant ailments, and the ability to limit physical activity. Dogs with one or more of the following conditions must be included in the high-risk group for thromboembolic complications: symptoms related to the disease (coughing, exercise intolerance, and ascites), abnormal chest X ray, high levels of circulating antigens, visualization of parasites by echocardiography, concomitant ailments, or the impossibility of limiting physical activity (435).

Supportive therapy is indicated for dogs with signs of cardiopulmonary dirofilariasis for which causal therapy is not recommended or for dogs before receiving adulticide or surgical therapy. This therapeutic approach includes the administration of drugs and/or the restriction of physical activity by crating (119). Corticosteroid use, such as prednisone at 1 mg/kg of body weight daily for 4 to 5 days, can control pulmonary inflammation and thromboembolic phenomena. Diuretics can be used to reduce pulmonary edema and pleural effusion when congestive heart failure is present, and digoxin is administered exclusively to control atrial fibrillation in severe cases. Oxygen therapy is advised when the animal exhibits respiratory difficulties.

Treatment with adulticides must be performed exclusively with melarsomine hydrochloride. The standard protocol dictates the administration of two intramuscular injections every 24 h in doses of 2.5 mg/kg. This two-injection protocol kills only about 90% of the adult worms. The three-dose alternate protocol (a single dose

followed by two injections, which are administered at least 30 days after the initial injection and 24 h apart) kills 98% of worms; besides, the first single dose of melarsomine eliminates 90% of male parasites and 10% of females, leading to a safe 50% reduction in the overall parasite load and thereby lessening the chance for embolic complications and allowing the recovery of the organism prior to the next 2-dose regimen, which will eliminate the rest of the adult worms (7). Adulticide therapy unavoidably leads to the development of pulmonary thromboembolism, especially in cases with high systemic levels of dead parasites. The risk of this condition can be reduced by restricting physical activity for 30 to 40 days following adulticide treatment and by administering heparin and glucocorticoids when necessary (433). It is known that some macrocyclic lactones have adulticide potential (259). Ivermectin has a partial adulticide effect if administered at a dose of 6 to 12 mg/kg of body weight monthly for 16 months, and the efficiency can reach 100% if treatment is extended to 30 months (259). Nevertheless, macrocyclic lactone administration is not recommended as the therapy of choice because the adulticide effect requires an extremely long treatment period. Throughout this period, the disease continues to progress, leading to damage to the dog's health and, potentially, the development of thrombi, which can occur unpredictably. Indeed, some researchers have observed that the health of animals treated with ivermectin for 24 months can worsen (438). Surgical therapy is performed on dogs with VCS using flexible alligator forceps introduced via the jugular vein. This instrument, guided with the aid of a fluoroscope, reaches into the right cardiac cavity and the pulmonary artery down to its lobar branches to extract the infective worms (187). The intraoperative mortality risk is very low, and survival and recovery rates are positively correlated with the number of parasites removed. Unlike adulticide treatments, the surgical extraction of filariae can potentially avoid the risk of the formation of a pulmonary thromboembolism (297).

Considering the severity of the disease and the difficulty and risks of therapy in infected dogs, prophylaxis is very important. The prophylactic treatment of choice in terms of safety and efficacy consists of the administration of macrocyclic lactones such as ivermectin, milbemycin oxime, moxidectin, or selamectin (205, 260, 268). Prophylaxis must start 1 month prior to the transmission period and finish 1 month after this period ends. In zones of endemicity or in regions where the climate allows transmission throughout the year, the continuous annual administration of prophylaxis is recommended. The above-mentioned drugs do not prevent the inoculation of larvae, but they do impede larval development. Testing for microfilaremia and circulating antigens is necessary in dogs undergoing chemoprophylaxis for the first time, in dogs over 1 year old without prior medical history, and at the beginning and end of each season in animals receiving routinely scheduled chemoprophylaxis (146). In addition, the American Heartworm Society recommends that animals receiving ongoing continuous chemoprophylaxis be tested once per year. Currently, researchers are debating whether the possible loss of efficacy of macrocyclic lactones is due to the development of resistance (57) or the improper use of the drug during treatment.

The prevention of patent infections by *D. repens* with macrocyclic lactones is questionable, and to date, only continuous-release moxidectin microspheres have demonstrated full efficacy in experimental studies (152).

The characteristics of feline cardiopulmonary dirofilariasis



complicate the diagnosis and assessment of the clinical status in many cases, hampering the selection of a treatment regimen. If the cat does not exhibit symptoms despite the presence of other evidence of dirofilariasis, adulticide treatment should be avoided, because cats can heal spontaneously. In such cases, examination of the patient at 6- to 12-month intervals, including repeated antigen and antibody detection and chest X ray, is recommended (307).

Adulticide therapy as performed on dogs (by the administration of melarsomine dihydrochloride) is not used on cats due to the elevated risk of pulmonary thromboembolism and potential anaphylactic-like reactions caused by the simultaneous death of the infecting parasites and the low therapeutic index of this product in cats. Thus, adulticide therapy is considered a treatment of last resort for cats in stable condition that present with clinical signs that a corticosteroid-based therapy cannot control. In addition, although feline infections are caused by a limited number of worms, the small volume of the right heart chambers and pulmonary arterial circuit favors the unexpected appearance of severe cardiopulmonary decompensation during the death of the adult worms, which can have serious and potentially fatal consequences. The literature on melarsomine-based therapy in cats is very limited to date. Preliminary data suggest that melarsomine is toxic to cats, even at dosages as low as 3.5 mg/kg of body weight (159, 267). Some studies reported 36% to 86% reductions in filariae depending on the dosage protocol (229, 238). For similar reasons, alternative treatments used on dogs, such as those based on 2-year ivermectin administration schedules, should also be excluded (435). Monthly ivermectin treatment by oral administration for 2 years at a dose of 24 µg/kg of body weight was shown to reduce the parasite load by 65% compared to that of untreated cats (169). Because most infected cats experience low parasite loads, the issue is not only the parasitic mass but also anaphylactic reactions that follow the death of the worms. This issue most likely occurs with ivermectin treatment as well, with the intensities of these potential reactions being unpredictable.

Surgical therapies are highly dangerous in cats, given the reduced dimensions of the feline jugular veins, which do not allow parasite retrieval from the right heart chambers without tissue damage in most cases. The accidental rupture of adult worms during surgical intervention due to these limitations may lead to the release of parasite products and anaphylactic shock, followed by death.

Due to the above-mentioned limitations of therapy, cats with symptoms of dirofilariasis should receive only corticosteroid-based supportive therapy to control the signs and symptoms of the infection while awaiting the completion of the parasite life cycle and spontaneous healing (439). The regression of radiographic signs and conversion to negative antigen test results are evidence of the spontaneous regression of the infection (307).

The administration of decreasing dosages of prednisone is usually effective in symptomatic and asymptomatic infected cats that exhibit radiographic evidence of disease and when antigen and/or antibody tests are positive. An initial daily dosage of 1 to 2 mg/kg of body weight every 12 to 24 h is recommended, gradually decreasing to 0.5 mg/kg every 2 days for 2 weeks, followed by observation with no treatment for an additional 2 weeks. At this point, the effect of treatment must be evaluated clinically and via chest X ray. This treatment regimen should be repeated in cats with recurring clinical signs (15).

Cats that exhibit acute signs must be stabilized quickly with the

appropriate supportive therapy to prevent shock. Depending on the particular circumstances, intravenous corticosteroids, electrolyte-balanced solutions, bronchial dilators, and oxygen can be applied. Diuretics are contraindicated, even for infected cats with severe interstitial or alveolar patterns. Aspirin and other non-steroidal anti-inflammatory drugs (NSAIDs) have not produced demonstrable benefits and can actually exacerbate lung parenchymal disease (15).

Monthly chemoprophylaxis is the only effective option for cats that reside in regions where canine dirofilariasis is endemic (52). Even cats that live exclusively indoors, according to their owners, are at risk for *D. immitis* infection. There are various medications for prophylaxis. Monthly oral doses of ivermectin or milbemycin oxime, topical selamectin, or moxidectin with the addition of imidacloprid are highly effective and safe for routine use (261). These drugs must be administered to all cats living in regions of endemicity during the entire transmission period of the disease, beginning at 8 weeks of age. Monthly dosages of preventive drugs are as follows: 24 µg/kg of body weight of ivermectin, 2 mg/kg of milbemycin oxime, 1 mg/kg of moxidectin, and 6 to 12 mg/kg of selamectin. The administration of these drugs to cats does not prevent antibody seropositivity. However, the use of preventive drugs does have certain advantages, including complementary activity against some intestinal parasites and, in the case of topical treatments, against ectoparasites. Annual administration is simple for owners and demonstrates retroactive efficacy as a safeguard against a missed monthly dose. As for dogs, prior diagnostic testing to rule out potential ongoing infections is recommended (146).

Regarding the treatment of ferrets, due to posttreatment thromboembolism with low survival rates, adulticide therapy is not an acceptable alternative in most cases (434). The best option for clinical management is prophylaxis with macrocyclic lactones (ivermectin, selamectin, or imidacloprid-moxidectin), which in various doses and with different routes of application have high therapeutic success rates (261, 434). More information can be found in several extensive reviews of heartworm disease in ferrets (258, 259, 261, 434).

Chemotherapy is not recommended for human dirofilariasis. When treated, surgery is usually used, in most cases due to the suspicion of a malignant origin of the nodule or the presence of worms in ocular locations. The removal of subcutaneous nodules or worms from the ocular conjunctiva is a simple procedure, but surgical intervention is much more complex for pulmonary, ocular, retro-ocular, or other internal locations (138, 208, 317). This intervention could be avoided in many cases of a lung location; thus, the most important point in the management of human dirofilariasis is its differentiation from other causes of nodules susceptible to being removed by surgery (391).

#### **Wolbachia as a Therapeutic Target**

The symbiotic relationship between *Wolbachia* and various species of filariae, including *D. immitis* and *D. repens*, has provided promising new options for the treatment of filariasis using *Wolbachia* as a therapeutic target (422). The objective of these strategies is to block filarial development (29) and decrease or eliminate the secondary reactions that are derived from bacterial elimination by the host organism when worms are destroyed by filaricides (216). Symbiotic *Wolbachia* bacteria are susceptible to tetracyclines, a finding which has inspired research on the effects of these antibi-

otics, alone or in combination with other drugs, on human and animal filariae (180, 261). Various studies using diverse experimental models, drugs, and treatment regimens have obtained significant, although somewhat discordant, results. Initial studies reported that tetracycline blocked the intrauterine development of *Brugia pahangi* and *D. immitis* microfilariae (29), dramatically depleting the *Wolbachia* population in filariae when applied for 2 weeks prior to the L4-to-L5 molting of *B. pahangi* in the Mongolian gerbil (*Meriones unguiculatus*) model, although a regrowth of bacterial populations was observed after 160 days (82). In contrast, Chirgwin et al. (90) did not find any changes in molting in the same model following tetracycline treatment, although the parasite load decreased after treatment. In BALB/c mice infected with *Litomosoides sigmodontis* and treated with rifampin alone or with doxycycline for 14 days, the elimination of *Wolbachia* from worms and the inhibition of development and embryogenesis were shown, and few worms survived after treatment (446). Microfilaricide and adulticide activity (163) and the total elimination of microfilariae for at least 4 months were found when tetracycline treatment was followed by the administration of acaciasides (*Acacia auriculiformis*-derived saponins) (104). The effects of antibiotic treatment on *D. immitis*-infected dogs have also been studied. Doxycycline in combination with ivermectin or ivermectin plus melarsomine lowered the incidence of inflammatory pulmonary lesions and thrombi compared with controls treated with either doxycycline or melarsomine alone (213, 261). Other studies have shown that interfering with dirofilariasis transmission using antibiotics is feasible (261, 363). Recently, research has focused on the potential mechanisms involved in the decreased production of microfilariae following *Wolbachia* depletion in filarial tissues after treatment with antibiotics (222). Those studies showed that the elimination of *Wolbachia* induces the extensive apoptosis of germ cells in adults and of somatic cells in embryos, microfilariae, and L4 larvae but not in hypodermal cords, suggesting that symbiotic bacteria regulate apoptosis in the nematodes that host them. Nevertheless, further research on the long-term administration of medications and the likelihood of *Wolbachia* population regrowth is needed to ascertain the efficacy of antibiotic treatment against dirofilariasis. The American Heartworm Society nonetheless recommends doxycycline therapy due to its beneficial effects. Research exploring the possibility of the administration of novel antibiotics and the identification of relevant genes and enzymes involved in *Wolbachia* physiology may reveal new targets for future clinical trials (180, 414).

#### THE HOST-PARASITE RELATIONSHIP IN DIROFILARIASIS

The host-parasite relationship in dirofilariasis is complex due mainly to (i) the capacity of *D. immitis* and *D. repens* to infect different hosts in which parasites achieve different levels of development and give rise to different pathologies and (ii) the presence of *Wolbachia* symbiotic bacteria in both the larvae and adult worms of both of the above-mentioned species, with hosts with dirofilariasis being exposed to antigens from both the nematodes and the bacteria (393). The types of responses induced by the two antigenic sets and the predominance of one over the other are related to the survival or death of the parasite and to the inflammatory response typically found in cases of dirofilariasis (390).

#### Immune Response: Basic Facts

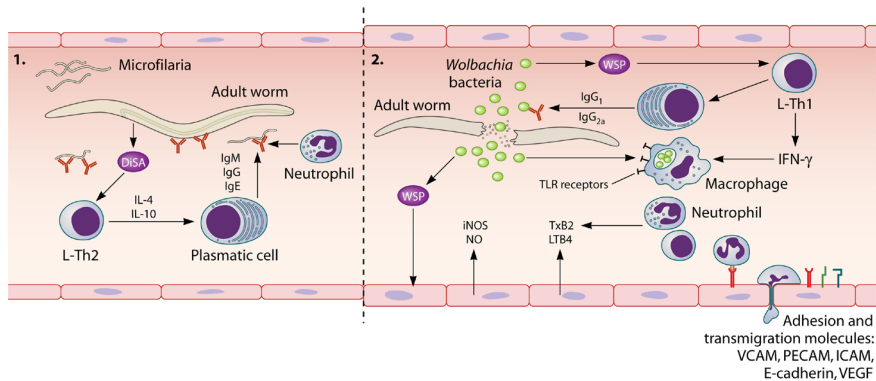
Before the discovery of *Wolbachia* and prior to considerations of the consequences of the presence of these bacteria, exceptional work that answered some important questions about the host-parasite relationship in dirofilariasis was performed with canine hosts and experimental models. Some of those data and conclusions were compatible with those obtained later, in which the existence of symbiotic bacteria represents a fundamental element of the research design. The immune response against *D. immitis* infections shows apparently contradictory data for dogs. The development from infective larvae to adult worms and the chronic nature of most of these infections seem to suggest a scarce efficiency of the immune response and the ability of the parasite to avoid the host's control mechanisms by reducing its immunogenicity or by inducing a state of immunotolerance in the host. However, it is generally accepted that the host is often able to control the worm burden and maintain it within limits compatible with its own survival, destroying most of the larvae acquired from reinfections (389). Different antibody isotypes (IgM, IgG, and IgE) against every developmental stage of the parasite are found, with the highest levels of antibodies being found in microfilaricidal infections (109, 145, 166–168, 272, 415). It has been postulated that antibody-mediated mechanisms are efficient in the elimination of microfilariae, with both IgM and IgG mediating neutrophil adhesion to the surface of microfilariae. This has lethal cytotoxic effects on larvae (447) yet is ineffective against adult worms. In chronic infections, a progressive suppression of cellular immunity is observed, with antibody responses being preserved (167, 449).

An increase in antibody responses after treatment with adulticides has been reported (416), and the subsequent massive antigen release after treatment correlates with increasing immunopathogenic responses (111). Currently, there is evidence that dead adult worms and microfilariae release *Wolbachia* symbiotic bacteria into the blood, which then interact with host tissues. IgG antibodies specific for the dominant *Wolbachia* surface protein (WSP) have been detected in the blood and urine of dogs with various clinical presentations of dirofilariasis (216, 288), in naturally and experimentally infected cats (38, 289), and in humans diagnosed with pulmonary and subcutaneous dirofilariasis (162, 396). In addition, immunohistochemical staining with polyclonal antibodies against WSP has revealed the presence of *Wolbachia* in multiple tissues and immune cells of dogs with heartworm disease (215, 216, 288). Likewise, individuals with *D. repens* subcutaneous dirofilariasis exhibited a strong anti-WSP IgG response and positive immunohistochemical staining for WSP in granuloma inflammatory cells (162).

#### Both the Presence of Microfilariae and the Clinical Status of Dirofilaria-Infected Hosts Influence the Intensity of the Antibody Response

As mentioned above, various experimental studies indicated that a strong antibody response against microfilariae occurs in dogs infected with *D. immitis*. Both anti-*D. immitis* and anti-*Wolbachia* IgG levels are significantly higher in microfilaricidal dogs than in dogs with amicrofilaricidal infections (167, 255, 292).

Researchers have also assessed differences in antibody levels and their association with the clinical status of the infected host. There have been reports describing amicrofilaricidal dogs with massive



**FIG 16** Predicted immune events occurring during *D. immitis* infections. (1) The Th2-type anti-inflammatory response is stimulated by *D. immitis* antigens and the presence of microfilariae. The expression levels of IL-4 and IL-10 are increased, and antibodies related to the Th2-type response arise: IgG1 (dogs) or IgE (humans). (2) The Th1-type proinflammatory response is stimulated by *Wolbachia* bacteria released from dying worms. The *Wolbachia* surface protein (WSP) stimulates the expression of IFN- $\gamma$  interacting with monocytes (probably through Toll-like receptors [TLR]) and inhibits their apoptosis. *Wolbachia* also stimulates the production of the Th1-type antibody response and the expression of proinflammatory mediators by vascular endothelial cells. Endothelial cells also increase the expression levels of adhesion/transmigration molecules (intercellular adhesion molecule [ICAM], platelet endothelial cell adhesion molecule [PECAM], and migration vascular cell adhesion molecule [VCAM]) and cellular proliferation molecules (E-cadherin and vascular endothelial growth factor [VEGF]). Some of these stimuli are also triggered by *D. immitis* somatic antigen (DiSA).

pulmonary thromboembolisms that exhibited stronger anti-*D. immitis* and anti-*Wolbachia* IgG antibody responses than those of asymptomatic amicrofilaremic dogs (390). Among dogs with glomerulonephritis and proteinuria linked to dirofilariasis, higher levels of anti-WSP IgG antibodies were detected in the urine of microfilaremic animals than in amicrofilaremic animals (288).

A strong IgG response against *D. immitis* and *Wolbachia* in cats with heartworm disease is also elicited (38, 346). In cats experimentally infected with L3 larvae, a moderate and short-term IgG response against L3 antigens develops at 0 to 2 m.p.i. (347), whereas IgG production in response to adult worm antigens is detected from 2 m.p.i. until 6 m.p.i. In cats treated with ivermectin at 30 d.p.i., anti-*D. immitis* IgG levels decreased after the elimination of larvae (from 45 d.p.i. onward), whereas anti-*Wolbachia* IgG levels continued to increase, consistent with the depletion of larvae and the release of *Wolbachia* into the host's body (346). Human patients diagnosed with pulmonary dirofilariasis exhibit IgG or IgM responses against somatic and E/S antigens of adult *D. immitis* worms (395), whereas IgE antibodies predominate in asymptomatic individuals (129, 374). Significantly, high levels of anti-WSP IgG have consistently been detected in patients with pulmonary dirofilariasis but not in asymptomatic seropositive individuals (396) or in patients with *D. repens*-associated subcutaneous dirofilariasis (162). All of these data suggest that the antibody response is linked to both the parasitological status and the clinical status of the host (216).

#### Immunopathogenic Mechanisms in Dirofilariasis

An analysis of immune responses in BALB/c mice immunized with antigenic extracts from *D. immitis* adult worms (DiSA), which simulate the death of worms in the pulmonary arteries, revealed a dual Th1/Th2 response at both the cytokine and antibody levels (Fig. 16). The Th2 response was directed mainly against *D. immitis* antigens, whereas the Th1 response was stimulated by *Wolbachia* (256). This polarization of the immune re-

sponse has also been shown for natural infections. A Th2 response characterized by high levels of interleukin 4 (IL-4) and IL-10 mRNAs and IgG1 production predominates in microfilaremic canine infections. On the other hand, a Th1 response characterized by a lack of IL-10 expression and elevated levels of expression of inducible nitric oxide (NO) synthase (iNOS) and IgG2 production occurs in amicrofilaremic canine infections (287). IL-10 expression is related to hyporesponsiveness in lymphatic filariasis (248, 316, 350), and the lack of a cellular response in helminth infections has been associated with Th3/TR1 cell types that produce the anti-inflammatory cytokines IL-10 and transforming growth factor beta (TGF- $\beta$ ) (125). These data suggest that, as for other filariases, circulating microfilariae in dirofilariasis can induce an immune bias toward nonresponsiveness (Th2) in dogs with heartworm disease, allowing adult worms to survive over the long term (292). Inflammation is a main and frequent consequence of dirofilariasis (324, 328, 390, 423). Many data confirm the link between *Wolbachia* and inflammation when adult worms die. Most patients diagnosed with pulmonary dirofilariasis demonstrate an exclusively IgG1-based (Th1) proinflammatory response to WSP (390, 396), whereas individuals exposed to *D. immitis* without pulmonary nodules exhibit a predominantly IgE-based (Th2) response (129, 344) that is specifically stimulated by both a galectin and an aldolase from the parasite but not by *Wolbachia* (344). In agreement with these data, the immunization of BALB/c mice with recombinant WSP induced the expression of iNOS and gamma interferon (IFN- $\gamma$ ) mRNA and the production of NO and IgG2a (Th1), which are related to macrophage-mediated proinflammatory responses (55, 287). Macrophages play a role in the activation and regulation of this innate response and link the innate and adaptive responses (421). Macrophages and neutrophils are present in canine *D. immitis* infections (216) and in cases of human dirofilariasis, and both cell types have been described to be the most abundant cell types in pulmonary and



subcutaneous nodules (328, 388). Together with neutrophils, the key role of macrophages in the inflammation associated with onchocerciasis and lymphatic filariasis has been demonstrated (61, 421). In this respect, some studies indicated that WSP induces the chemotaxis of neutrophils (37) and can inhibit their apoptosis *in vitro*, which could contribute to the extension of the inflammatory state (36). Moreover, the presence of *Wolbachia* or of its molecules in the cytoplasm of leukocytes of dogs and humans with cardiopulmonary or subcutaneous dirofilariasis has been shown by immunohistochemistry (162, 216) (see Fig. S3 in the supplemental material). Some other observations, such as the downregulation of intravascular pulmonary macrophages in cats with live worms, in contrast to the upregulation of macrophages in those with dead worms, link the release of *Wolbachia* antigens to the presence of proinflammatory macrophages (118). Interactions between *Wolbachia* molecules and macrophages occurring through the Toll-like receptor (TLR) family (TLR2, TLR4, or TLR6) have been shown in cases of onchocerciasis and filariasis (60, 429). These findings suggest that *Wolbachia* interacts with macrophages via the same receptors as those for lipopolysaccharides (LPSs), which are molecules that are usually involved in bacterium-driven inflammation (421) and that *Wolbachia* does not possess (454).

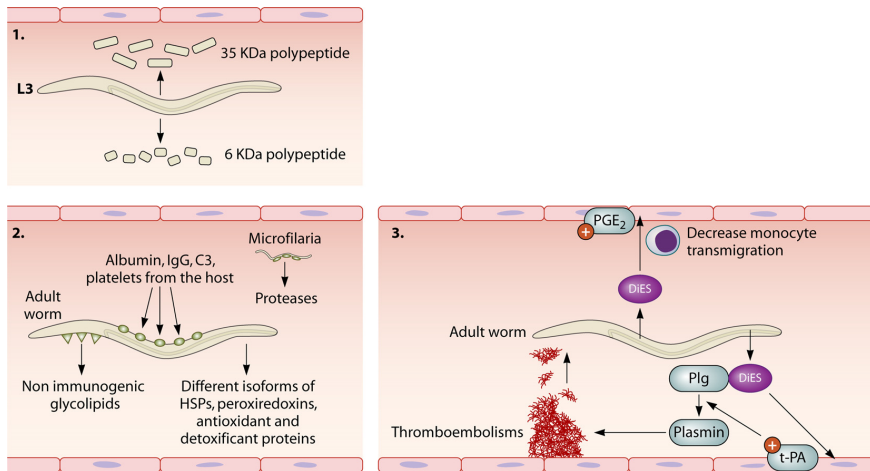
Other data that demonstrate the participation of *Wolbachia* in inflammation during dirofilariasis have been gathered by analyzing eicosanoid production during natural infections with *D. immitis*. Eicosanoids are lipid mediators derived from arachidonic acid that intervene in the regulation of inflammatory processes and modulate interactions between blood cells and endothelial cells (241). In experimental feline infections, both thromboxane B2 (TxB2), which stimulates vasoconstriction, platelet aggregation, and the proinflammatory Th1 response, and leukotriene B4 (LTB4), which is active in chemotactic and leukocyte activation (369), reach their maximal levels at 180 d.p.i., when inflammatory and obstructive reactions begin as preadult worms reach the pulmonary arteries (295). TxB2 is present at high levels in both feline and canine chronic infections and in human patients with pulmonary dirofilariasis but not in exposed asymptomatic individuals. In all cases, the presence of TxB2 correlates with elevated levels of anti-WSP IgG (291, 293, 295). In this respect, a considerable increase in intravascular TxB2 levels during septic shock has been demonstrated (27). All of these studies suggested that TxB2 production during dirofilariasis may be associated with the release of *Wolbachia* upon parasite damage and death.

As mentioned above, the vascular endothelium is altered during *D. immitis* infection, undergoing inflammation and endothelial cell disorganization (394). Vascular endothelial cell cultures stimulated with WSP exhibit significant increases in the expression levels of enzymes responsible for eicosanoid synthesis (cyclooxygenase 2 [COX2] and 5-lipoxygenase [LO]) and of TxB2 and LTB4 (296, 394), which suggests that symbiotic bacteria stimulate proinflammatory eicosanoid production in the vascular endothelium. In endothelial cells activated by *Wolbachia* antigens, increased expression levels of molecules associated with leukocyte adhesion and migration, including vascular cell adhesion molecule (VCAM), intercellular adhesion molecule (ICAM), and platelet endothelial cell adhesion molecule (PECAM); cell proliferation (E-cadherin and vascular endothelial growth factor [VEGF]); iNOS; and endothelial nitric oxide synthase (eNOS) have also been shown. Nevertheless, the facts that many of these molecules are also produced by endothelial cell cultures treated

with DiSA (394) and that filariae such as *Loa loa* that lack *Wolbachia* also induce inflammation suggest that *Wolbachia* itself is only partially responsible for the inflammatory responses triggered by filariasis and that parasite molecules may also participate in this process.

### Pathogenic Mechanisms Unrelated to the Immune Response

The concept that filariae show pathogenic mechanisms mediated by adult worms that affect endothelial cells was formulated by a multidisciplinary research team at Michigan State University in the 1980s and 1990s. Such mechanisms are thought to be due to excretions by filariae that alter the physiological functions of endothelial cells but not of arterial smooth muscle cells, thereby altering the capacity of the vascular wall to contract and relax. Those authors also suggested that the derived altered dilation of major arteries could explain, at least in part, the inability of dogs infected with *D. immitis* to adapt to circulatory demands during physical exertion (193). Data obtained by these and other researchers in multiple studies support this hypothesis. In healthy dogs, femoral artery stimulation by acetylcholine treatment produces increased guanylate cyclase levels in vascular smooth muscle cells and results in relaxation due to a non-prostaglandin-derived factor released by vascular endothelial cells. In contrast, in *D. immitis*-infected dogs, these relaxation mechanisms in response to acetylcholine differ, since at least two relaxation factors derived from endothelial cells are seemingly involved in the process. The most important factor is a prostaglandin synthesized through the cyclooxygenase pathway, and the second factor functions via vascular smooth muscle GMP (cyclic GMP [cGMP]). These results suggest that *D. immitis* secretes pharmacologically active factors that can alter endothelial cell function (193). Later studies provided evidence that products of the cyclooxygenase pathway in filariae, such as prostaglandin D2 (PGD2), induce the depression of endothelium-dependent relaxation at low, but not at high, acetylcholine concentrations (192). Changes observed for the arterial wall *in vivo* are short-lived and are completely dependent upon the presence of high concentrations of molecules released by female parasites (425). Endothelial cell-dependent relaxation was analyzed with and without inhibitors of NO synthase, cyclooxygenase, and guanylate cyclase. Responses to vasoconstrictive agents such as methacholine, substance P, and A-23187 were suppressed in the pulmonary arteries of dogs with dirofilariasis compared with healthy controls, indicating that behavioral changes in endothelial cells, but not smooth muscle cells, contribute to vessel relaxation. It has also been shown that histamine, which causes vasoconstriction in healthy dogs, induced vasodilation in the arteries of dirofilariasis-infected dogs, which switched to vasoconstriction when arterial endothelial cells were eliminated (194). More recently, Kitoh et al. (202) identified specific molecules in adult somatic antigens of *D. immitis* responsible for the shock induced by *D. immitis* products. One of the identified molecules led to vasoconstriction through a direct action on the smooth muscle in the vascular wall, and a second one induced vasodilation as a consequence of NO production by endothelial cells. All of these data are consistent with the hypothesis that the interaction of *D. immitis* products with vascular endothelial cells causes alterations in the arterial dilation response in dogs with heartworm disease.



**FIG 17** Mechanisms of survival and immune evasion in *D. immitis*. (1) Short-term immune evasion by L3 larvae. Infective larvae avoid the host immune response, releasing large amounts of two surface antigens, of 6 and 35 kDa. (2) Long-term immune evasion. Preadult/adult worms mask their surface, adsorbing different host molecules and cells. They have surface nonimmunogenic glycolipids and many isoforms of heat shock proteins (HSPs) and detoxificant enzymes that eliminate toxic products synthesized by macrophages and neutrophils. Microfilariae have surface proteases that digest host antibodies. (3) Modulation of the vascular environment. Live adult worms release metabolic products (E/S antigens) that stimulate anti-inflammatory prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) eicosanoid and decrease monocyte transmigration. Additionally, E/S antigens bind plasminogen and activate plasmin to eliminate thromboembolisms in the presence of the tissue plasminogen activator (t-PA), whose expression is also stimulated by E/S antigens in vascular endothelial cells. DiES, *D. immitis* excretory/secretory products; PLG, plasminogen.

### Parasite Survival Mechanisms

Because many of the threats to the survival of *D. immitis* worms are of immunological origin and are derived from the degradation of the vascular environment, the parasite must trigger mechanisms to both evade immunity and retard arterial damage. Existing data demonstrate that *D. immitis* deploys several different immune evasion strategies in each of its developmental stages (Fig. 17) (389). Both *in vivo* and *in vitro* evidences indicate that L3 larvae, quickly transforming to L4 larvae, eliminate between 10% and 20% of their surface antigens, constituted primarily of two molecules of 35 kDa and 6 kDa. These antigens are not replenished, allowing the larvae to maintain a low antigenic profile that is difficult for the immune system to detect (184). This observation is consistent with the low intensity and short duration of antibody responses to L3 antigens in dogs (347). Adult worms also seem to reduce their immunogenicity by preventing the exposure of glycosylation sites on their external surface glycoproteins, as mentioned above. This structural characteristic may constitute a specialized way of protection against host immune responses. Adult worms have developed additional long-term immune evasion mechanisms. The external surface of *D. immitis* adults can capture platelets and adsorb albumin, IgG, and complement fraction 3 (C3) in dogs (48, 190), thereby masking the worms and preventing the recognition of cuticular antigens. A nonimmunogenic 6- to 10-kDa glycolipid associated with the surface of adult worms may constitute a barrier against protease activity at the epicuticle-host interface, also contributing to the evasion mechanisms (381). Microfilariae are also able to evade immune responses. They present surface-linked proteases that lyse epicuticle-binding IgG antibodies, which suggests that microfilariae can avoid host humoral responses (415). In addition, both *D. immitis* and *D. repens* produce

numerous proteins that may be related to survival, such as heat shock proteins and enzymes with antioxidant or detoxification and glycolytic functions, all of which have been linked to stress responses and the ability of parasites to interfere with reactive oxygen species released by macrophages and neutrophils (157). It is unknown to what extent antibodies against parasite molecules can block the parasites' activity during a natural infection and thereby limit the utility of parasite survival mechanisms. Nevertheless, the presence of various isoforms of these proteins seems to indicate that *Dirofilaria* species possess redundant biochemical systems that make the deleterious effects of the immune response more difficult to be reached.

Few other studies suggested the participation of some E/S antigens in mechanisms linking *D. immitis* with its vascular environment. Endothelial cell cultures stimulated with DiE/S showed a significant increase in prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) levels and a significant decrease in monocyte transmigration compared with unstimulated cultures (290). PGE<sub>2</sub> stimulates anti-inflammatory activity (43), induces vasodilation, and decreases vascular permeability (50), suggesting that DiE/S can modify the vascular environment and limit the damage from inflammation in the perivascular spaces. Moreover, cats experimentally infected with *D. immitis* showed the highest serum PGE<sub>2</sub> levels at 60 d.p.i., followed by a dramatic decrease, associating high PGE<sub>2</sub> levels with parasite survival during the critical migration phase of the parasite (295). PGE<sub>2</sub> has also been detected during different developmental stages of filariae in humans and in monocytes from hyporeactive individuals with onchocerciasis (62, 63, 242), with its detection being related in all of those cases to the containment of inflammation.

Noteworthy, it has been shown that DiE/S activates the fibrino-

TABLE 4 Key aspects of dirofilariasis in different hosts<sup>a</sup>

Aspect of dirofilariasis	Host		
	Dogs	Cats	Humans
Biological features	Mf <sup>+</sup> or Mf <sup>-</sup> infections Sexual maturity at 4 m.p.i. Adult worms live over 7 yr Low to high parasite burden	Mf <sup>-</sup> infections Sexual maturity at 8 m.p.i. Adult worms live a maximum of 2 yr Few worms (1 to 3 worms)	Mf <sup>-</sup> infections No sexual maturity in <i>D. immitis</i> infections Generally 1 immature worm Quite mature worms are detected in <i>D. repens</i> infections
Immunology	Mf <sup>+</sup> infections; Th2-type response  Mf <sup>-</sup> infections; Th1-type response  IgG antibody response against <i>Wolbachia</i> can be present	Low antibody response against L3 antigens  Strong antibody response against adult worm antigens and <i>Wolbachia</i>	Th1-type response against WSP in patients with pulmonary nodule  IgE (Th2) antibody response in healthy exposed individuals
Clinical aspects	Most infections have chronic development Simultaneous deaths of many worms can cause acute episodes Most symptoms related to respiratory and cardiac alterations	Less predictable evolution of disease than in canine infections Respiratory, gastrointestinal, cardiovascular, and neurological signs	Benign pulmonary or subcutaneous nodules that can be confused with cancer Most pulmonary cases are asymptomatic; when they appear, they are nonspecific Serious ocular cases are appearing with increasing frequency
Diagnosis options	Knott test  Circulating antigen detection tests  Image diagnostic techniques	Antibody or antigen tests together with image diagnostic techniques	Detection by causes unrelated to <i>Dirofilaria</i>  Histology, serology, and molecular techniques Direct or indirect detection of <i>Wolbachia</i> can be a complementary tool
Treatment	Adulticide treatment with melarsomine hydrochloride, simultaneous doxycycline treatment to decrease <i>Wolbachia</i> levels Prophylaxis with macrocyclic lactones  Surgical therapy in cases of VCS	Adulticide therapy is the last resort in cats with clinical signs  Prophylaxis with macrocyclic lactones	No chemotherapy treatment  Surgical removal of nodules or worms when necessary

<sup>a</sup> Mf<sup>+</sup>, microfilaremic; Mf<sup>-</sup>, amicrofilaremic; WSP, *Wolbachia* surface protein; VCS, vena cava syndrome.

lytic system *in vitro* and binds plasminogen, resulting in plasmin generation in a manner that is dependent on tissue plasminogen activator (t-PA). Vascular endothelial cells stimulated with DiE/S significantly increased their t-PA expression levels, thus potentially involving the vascular endothelium in the fibrinolytic activity of some of the worm molecules. The plasminogen-binding capacities of several molecules present in DiE/S, such as HSP60, actin, GAPDH, galectin, and P22U, have been demonstrated (156). These data indicate that some metabolic products of adult *D. immitis* worms can activate thrombus removal, one of the most severe complications of cardiopulmonary dirofilariasis, in which the vascular endothelium plays a key role. Given that t-PA overexpression in the damaged endothelium induces the proliferation and migration of smooth muscle cells in humans (457), the possibility that the proliferative endarteritis that develops during dirofilariasis is related to this mechanism is worth investigation.

#### CONCLUSIONS AND FUTURE TRENDS

The presence of two different species, *D. immitis* and *D. repens*, that infect three main hosts, humans, dogs, and cats, resulting in different pathologies, and the presence of several wild reservoirs potentially interacting with the anthropogenic environment con-

fer a high level of biological, clinical, and epidemiological complexity to dirofilariasis (Table 4). There are clear imbalances in the knowledge of the different elements of this parasitological mosaic. Human dirofilariasis is likely the branch with the greatest dearth of knowledge. Issues such as the small number of cases that develop pulmonary or subcutaneous nodules and the apparent differences in the capacities for the development of *D. immitis* and *D. repens* in humans must be examined.

From an epidemiological perspective, dirofilariasis is considered an emergent parasitic disease of humans and animals. Rapid and significant changes in the distribution and prevalence of canine reservoirs are being reported around the world, and these changes in turn change the epidemiological parameters for human and feline dirofilariasis. Global warming influences the stages of the parasite life cycle that take place in vectors. This, together with pet management and human intervention in the environment that affect vector and vertebrate hosts, could account for substantial increases in numbers of dirofilarial infections in the near future. This has already resulted in more frequent reports of cases of dirofilariasis, which, together with its severity in some human cases, especially in cases of subcutaneous/ocular dirofilariasis in some European regions, has raised concern about and in-

terest in this disease and heightened vigilance in recent years among physicians and researchers. GIS and RS techniques are contributing to the precise determination of how dirofilariasis is distributed and should be used for the reliable prediction of its changes and the potential emergence of new areas of endemicity, making these techniques invaluable for the design of parasite control measures.

Knowledge of the genome, proteome, biochemistry, metabolism, molting, pathogenesis, and survival mechanisms of *D. immitis* and *D. repens* is limited to date. Further in-depth studies of these topics may provide information on novel therapeutic and infection control targets and explain the differences in the relationships between these parasites and their different hosts.

New tools that allow the acquisition of objective parameters to evaluate and describe the damage in infected animals are being developed. These tools should contribute to improve prognosis and thus to define appropriate guidelines for the clinical management of dirofilariasis in different hosts.

Knowledge of the role of *Wolbachia* in immune and immunopathogenic mechanisms of dirofilariasis and in treatment constitutes part of the most significant advancement in dirofilariasis research, e.g., by giving clues to improve the quality of life of treated animals by minimizing the inflammatory side effects of treatment. Research on additional antibiotics and routes of administration will most likely lead to improvements in treatment outcomes. Moreover, research that extends our knowledge of the symbiotic relationships between *Wolbachia* and filariae may yield further information on how to manipulate these relationships to avoid parasite development. This should not preclude further studies focused on *Dirofilaria* sp. antigens, since the available data suggest that they play important roles not only in parasite survival but also in pathogenic processes.

Research on the development of vaccines for dirofilariasis has not progressed since the first tests were performed with irradiated larvae (393). The great molecular complexity and large number of isoforms of many antigens and the lack of immunogenicity of some proteins in natural infections are issues that require further investigation. This knowledge will enable researchers to perform protection studies with a more rational approach aimed toward interfering with multiple key processes in the life of the parasite simultaneously.

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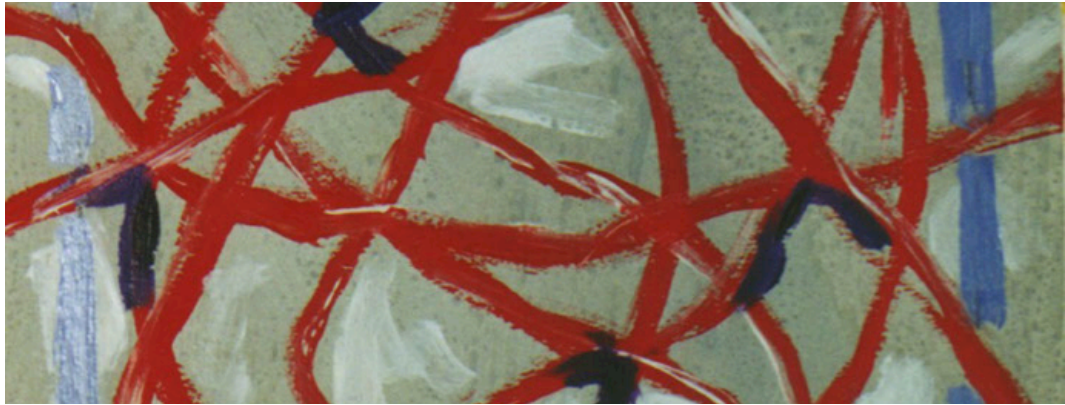


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## ARTÍCULOS

Montoya-Alonso JA, Morales M, Ferrer O, Molina JM & Corbera JA  
**The prevalence of *Dirofilaria immitis* in Gran Canaria, Canary Islands, Spain (1994-1996).**  
*Veterinary Parasitology*, 75,221-226 (1998).

Montoya-Alonso JA, Morales M, Juste MC, Bañares A, Simon F & Genchi C.  
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Montoya-Alonso JA, Carretón E, Morchón R, Silveira-Viera L, Falcón Y & Simón F  
**Current epidemiological status of *Dirofilaria immitis* in the pet population of the Canary Islands.**  
*Veterinary Parasitology*, 2015-2016 (submitte).







### **OBJETIVO 1**

Determinar la prevalencia de *Dirofilaria immitis* en perros de la isla de Gran Canaria los años 1994,1995 y 1996.

### **ARTÍCULO**

**The prevalence of *Dirofilaria immitis* in Gran Canaria, Canary Islands, Spain (1994-1996).**  
*Veterinary Parasitology*, 75,221-226 (1998).





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## The prevalence of *Dirofilaria immitis* in Gran Canaria, Canary Islands, Spain (1994–1996)

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### Abstract

Blood samples from 2034 dogs were tested to detect *Dirofilaria immitis* antigen during three consecutive years (from 1994 to 1996) in Gran Canaria Island, Canary Islands, Spain. The prevalence of heartworm infection was 67.02% in 1994, 58.92% in 1995 and 52.18% in 1996, with a mean prevalence of 58.89%. Heartworm infection was more common in males (56.19%) than in females (43.81%), in dogs aged between 3 and 6 years old. The distribution of the disease in the different climatic zones was studied. Chronological changes in the dog's prevalence for heartworm infection in the three consecutive years and the role of the epidemiological factors in the changes of the positive rates were evaluated. © 1998 Elsevier Science B.V.

*Keywords:* Dog; *Dirofilaria immitis*; Heartworm disease; Epidemiology–Nematoda; Canary Islands

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### 1. Introduction

Dirofilariosis is a parasitic disease, transmitted by a mosquito and can cause cardiopulmonary disease. The etiologic agent is *Dirofilaria immitis*, one of the most pathogenic nematode parasites of the dog. It is spread by many different species of mosquitoes, and the geographical extent of the disease is directly related to that of susceptible mosquitoes. Female mosquitoes serve as the intermediate host by obtaining a blood meal from a dog with circulating *D. immitis* microfilaria. Heartworm inhabits the right ventricle and pulmonary arteries of dogs and other animals. The typical changes of

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this disease include right ventricle enlargement, enlargement of the main, lobar and peripheral pulmonary arteries, and perivascular parenchymal disease.

Dirofilariosis is a disease of world wide distribution, but the most endemic areas are those with temperate, tropical and subtropical climates in the planet; where mosquito populations are high and stable. Other regions with cold weather, with hot summers and with rivers, lakes and wide irrigation lands are also suitable for the development of the disease. Frequency of infection is related to life style, with male dogs being more frequently than female dogs.

In recent years, several epidemiological studies have been performed in different countries. Although heartworm disease is distributed widely in the world, the higher prevalences appear in South America, Central America and Caribe, North America, Mediterranean countries, Polynesia, Australia and Japan.

Spain, belonging to the mediterranean countries, is one of the enzootic regions of *D. immitis*. The prevalence of the disease is high in the south of the Iberian Peninsula and in the Canary Islands (Valladares et al., 1987; Anguiano et al., 1985; Guerrero et al., 1989; Ortega-Mora et al., 1988, 1991; Castillo et al., 1989; Pérez et al., 1989; Rojo-Vazquez et al., 1990; Gutiérrez et al., 1995). Canary Islands, situated in the 28th parallel, on the African Atlantic coast has the highest prevalence in Spain, but only a few surveys has been performed in Canary Islands in the past (Guerrero et al., 1989). Most veterinarians practice in Canary Islands include heartworm disease in the differential diagnosis of patients with clinical signs referable to the lungs and heart. Our objective was to determine the prevalence of *D. immitis* in dogs in Gran Canaria Island, from 1994 to 1996.

## 2. Materials and methods

We studied 2034 dogs (1143 males and 891 females) for *D. immitis* infection over three consecutive years (1994 to 1996), 567 dogs (306 males and 261 females) were analysed in 1994, 779 (441 males and 338 females) in 1995 and 688 (396 males and 292 females) in 1996.

Random subjects of the canine population brought to the veterinary clinics on Gran Canaria Island for routine health care examinations or procedures were included in the survey. All dogs were 6 months old or older, and many breeds were represented. None of the dogs in the study received heartworm chemoprophylaxis or chemotherapy. The dogs were randomly selected by the veterinarians, and the samples were obtained by cephalic venipuncture and kept refrigerated until assayed in our laboratory. A record form was completed for each dog, giving pertinent identification, history and demographic data. Blood was remitted to our laboratory where a haemagglutination test (Vetred *Dirofilaria*<sup>®</sup>, Rhône Mérieux) was used for serologic detection of *D. immitis* antigens.

Gran Canaria Island is an approximately round island of 50 km in diameter, with four isoclimate zones: a zone with a dry and stepparic climate (DS), where the average temperature is higher than 18°C and with a dry summer; a zone with a dry and desertic climate (DD) where precipitation is lesser than 18 mm, temperatures higher than 18°C

Table 1  
Sex distribution of dogs with *D. immitis* infection

Sex	1994			1995			1996			Mean		
	No. of animals	No. of positives	Rate (%)	No. of animals	No. of positives	Rate (%)	No. of animals	No. of positives	Rate (%)	No. of animals	No. of positives	Rate (%)
Males	380	208	54.74	459	256	55.77	359	204	56.82	1198	668	55.76
Females	380	172	45.26	459	203	44.23	359	155	43.18	1198	530	44.24
			**			***			***			***

\*\* =  $p < 0.01$ ; \*\*\* =  $p < 0.001$ .

and a very dry summer; a zone with a temperate climate but with a hot and dry summers and mild winters (TM); and a zone with a temperate climate but with cold winters and summers with a medium temperature below +22°C (TC).

The results were computed using the SAS statistical package. For each variable and class, means and statistical signification were computed. The means were compared among classes by means of the Student–Newman–Keuls test.

### 3. Results

Of the 2034 dogs, 1143 (56.2%) males and 891 (43.8%) females. The sex distribution of dogs in each year was (53.96% males and 46.04% males) in 1994; (56.61% males and 43.39% females) in 1995; and (57.55% males and 42.45% females) in 1996. Of the 2034, 28.51% of dogs ranged in age between 6 months to 3 years old, 48.18% between 3 and 6, and 23.31% were older than 6 years. Dogs distribution within the different isoclimate included 707 dogs (34.8%) in the DD zone, 617 (30.3%) in the TM zone, 442 (21.7%) in the TC zone and 268 (13.2%) in the DS zone.

The average seroprevalence of the disease in the three years studied was 58.89% (1198/2034). The prevalences of the disease in each year were 67.02% (380/567) in 1994; 58.92% (459/779) in 1995; and 52.18% (359/688) in 1996.

A significant difference was found between males and females affected dogs, more males than female dogs were affected ( $p < 0.001$ ) (Table 1).

Age distribution of the positive dogs is detailed on Table 2.

The average prevalences in the different breeds were as follows; Bardino (69, 23%), Presa canario (64, 76%), Podenco canario (63, 84%), Large crossbreed (58, 25%),

Table 2  
Age distribution of dogs with *D. immitis* infection

Age groups	1994			1995			1996			Mean		
	No. of animals	No. of positives	Rate (%)	No. of animals	No. of positives	Rate (%)	No. of animals	No. of positives	Rate (%)	No. of animals	No. of positives	Rate (%)
0.5–3 yr	380	73	19.21	459	92	20.04	359	60	16.71	1198	225	18.78
3–6 yr	380	198	52.10	459	252	54.90	359	195	54.31	1198	645	53.84
> 6 yr	380	109	28.68	459	115	25.05	359	104	28.96	1198	328	27.37

Table 3  
Isoclimate zone distribution of *D. immitis* in Gran Canaria

Climate	1994			1995			1996			Mean		
	No. of animals	No. of positives	Rate (%)	No. of animals	No. of positives	Rate (%)	No. of animals	No. of positives	Rate (%)	No. of animals	No. of positives	Rate (%)
DD	133	62	46.62	306	111	36.27	268	87	32.46	707	260	36.77
			NS			***			***			***
DS	91	72	79.12	100	80	80	77	55	71.42	268	207	77.24
			***			***			***			***
TM	196	133	67.86	212	146	68.87	209	130	62.20	617	409	66.28
			***									***
TC	147	113	76.87	161	122	75.78	134	87	64.92	442	322	72.80
			***			***			***			***

Medium crossbreed (45, 08%) and German Shepherd (41, 74%), (only breeds representing more than 5% of total dogs are included).

Table 3 shows the distribution of the different isoclimatic zones of the affected dogs in Gran Canaria. The Dry and Stepparic climate (DS) had the highest prevalence 77.24% (207/268) ( $p < 0.001$ ), and the Dry and Desertic climate (DD) had the lowest prevalence 36.77% (260/707) throughout the study.

#### 4. Discussion

Our present survey carried out in Gran Canaria Island (Canary Islands) reveals the highest prevalence of dirofilariosis ever reported in Spain, in contrast to Guerrero et al. (1989) who reported Huelva as the province of Spain with the highest prevalence (36.7%). In Guerrero et al. study the prevalence of Las Palmas de Gran Canaria was 36%.

Our prevalence between 52.18% and 67.02% is similar to those described in Cuba (Dumenigo et al., 1988) and Japan (Suenaga and Kitahara, 1978; Tanaka et al., 1985; Hatsushika et al., 1992); although we have never found prevalence as high described in Papua New Guinea (86%) by Hamir and Onaga (1986).

We observed some decrease in the prevalence of heartworm infection from 1994 (67.02%) to 1996 (52.18%). In the last year (1996), no statistically significant difference was found between positive and negative dogs. This is probably due to education of pet owners and the increased use of chemoprophylaxis.

Sex distribution of affected dogs coincide with the sex distribution in the population, but there were significant differences in male and female prevalences in the three years studied. This is similar to Selby et al. (1980), who found that non-castrated male dogs had the highest relative risk. In Gran Canaria, more male dogs live in the open air, due to their use in defence of property. They are, therefore, more likely to be bitten by mosquitoes. For many authors (Selby et al., 1980; Beugnet et al., 1994), age is an important risk factor, determined by time of exposure in the endemic area. Therefore,

older dogs have a higher prevalence of dirofilariosis than younger dogs. We found the highest prevalence in animals between 3 and 6 years old (53.85% average), and the lowest in animals under 3 years of age (18.78%).

Most hunting and farming breeds in our survey were affected. This is probably, because these dogs are maintained primarily outdoors in rural areas, and have low chemoprophylaxis, general and nutritional care. The most affected breeds were Canarian native dogs (Presa canario, Bardino and Podenco canario), which represent more than 55% of the canine population studied. Presa canario and Bardino canario are custody and defence breeds, which live outdoors, in gardens, without protection against vectors. For this reason, the prevalence of *D. immitis* in these breeds is high (64.73% and 69.23%, respectively). German Shepherds, commonly live outdoors, but usually owners use chemoprophylaxis and the prevalence decreased to 41.74%. In all breeds, prevalence decrease gradually year after year.

Weather is a critical factor in the prevalence of the disease. Transmission depends on the intermediate host, which have certain climate requirements (high relative humidity and higher than 15°C average temperature). Gran Canaria Island perfectly meets these climate requirements. DD (dry and desertic) zone, with very low rainfall and non-existence of water, lakes or rivers, has the lowest prevalence of Gran Canaria Island. In eastern Gran Canaria, Alisios winds blow continuously, making mosquito survival more difficult. Cities and villages are found mainly in the DD zone, and the majority of the canine population is under chemoprophylaxis.

DS (dry and stepparial) zone had the highest prevalence. All the farming activity of the island is developed in this zone, with stagnant water, hot weather with suitable temperatures for development of mosquito (annual average temperature higher than 18°C, in northern or north eastern coastal areas). Small villages and farm houses with dogs living outdoor predominate in this zone. Prevalence in TM zone (temperate climate, with hot and dry summers and mild winters) is also very high, probably due to available water and moderate temperatures. Chemoprophylaxis is not commonly used in many villages, although prevalence decreased in 1995 and 1996. The centre of Gran Canaria is located over 800 m above sea level, has a warm and regular climate, with high relative humidity. The prevalence of dirofilariosis in this area remains constant with inter annual variation below 7 points (Table 3).

The importance and severity of dirofilariosis in Gran Canaria Island has been corroborated as the highest ever found in Spain. Hopefully, this data will encourage prophylaxis and result in a decreasing prevalence of *D. immitis* infection in Gran Canaria. New surveys must be done in coming years to verify the efficiency of this effort.

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## **OBJETIVO 2**

Conocer la prevalencia de *D. immitis* en perros de la isla de Tenerife y las variaciones en función de los diferentes isoclimas de la isla (años 2002-2003).

## **ARTÍCULO**

**Seroprevalence of canine heartworm disease (*Dirofilaria immitis*) in Tenerife Island: An epidemiological update.**

*Parasitology Research*, 100 (1): 103-105, 2006.



# Seroprevalence of canine heartworm disease (*Dirofilaria immitis*) on Tenerife Island: an epidemiological update

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**Abstract** Blood samples from 823 dogs were tested for circulating *Dirofilaria immitis* antigen during a 1-year period (May 2002 to May 2003) on Tenerife Island, Canary Islands, Spain. Seroprevalence of heartworm infection was 21%. Heartworm infection was similar in males and females and was more common in dogs aged >6 years. Distribution of infection in varying climatic zones was not statistically different.

## Introduction

Heartworm (HW) disease is a cosmopolitan parasitic infection of domestic and wild carnivores, caused by the filarial nematode *Dirofilaria immitis*. The adult parasite is located in the pulmonary arteries and right cardiac chambers, and infection causes severe to very severe

disease in dogs and cats living in endemic areas. Areas with temperate, tropical, and subtropical climates, where mosquito populations are abundant and stable and where an active reservoir is present, are highly endemic. The frequency of infection is also related to the lifestyle of dogs and cats. In the same area, animals living outdoors show higher prevalence values than indoor dogs and cats (Glickman et al. 1984; Kramer and Genchi 2002).

In Spain, high infection prevalence has been found mainly in southern regions (Pérez-Sánchez et al. 1989; Ortega-Mora et al. 1991; Guerrero et al. 1995). However, the highest HW prevalence was found in Canary Islands located at the 28th parallel on the African Atlantic coast (higher than 50%; Montoya et al. 1998). The aim of this survey was to update HW prevalence in dogs and to evaluate if the climatic factors that characterize Tenerife Island can influence the infection prevalence in dogs. Tenerife has three main isoclimatic zones: 1) a zone with a temperate climate, hot and dry summers and mild winters (TM zone), 2) a zone with temperate climate and cold winters (TC zone), and 3) a zone with a dry, desert climate and very dry summers (DD zone) (Table 1; data from Instituto de Astrofísica de Canarias).

## Materials and methods

Eight hundred and twenty-three privately owned dogs presented to veterinary clinics from May 2002 and May 2003 for routine health examinations and procedures were included in the study. The criteria for inclusion were >6 months of age, no HW chemoprophylaxis, no previous history of heartworm infection and owner consensus to participate in the survey. Blood samples were obtained by cephalic venipuncture and were kept at +4°C until tested in

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**Table 1** Climate characteristics of Tenerife Island (Spain)

	Climatic zones		
	Temperate/mild	Temperate/cold	Dry/desert
Altitude m/sl	North: 0–800 South: 500–1,100	North: >800 South: >1,000	0–600
Annual mean temperature °C min–max	20.1 16.6–23.5	16.1 11.7–20.6	19.9 17–22.9
Annual rainfall mm	500	464	280
Annual mean relative humidity %	70.8	68.4	64.2

the laboratory. A complete record was kept for each dog, including identification (age, sex, breed), clinical history, and demographic data. Sera were examined using an ELISA test kit (Canine Heartworm Antigen, PetCheck PF IDEXX, Westbrook, Maine, USA) for detection of HW infection in dogs following manufacturer instructions.

The results by age, sex, breed, and sampling zone were analyzed using the SAS statistical package (13.0). The prevalence values were compared by Chi-square test.

## Results

Of 823 dogs, 406 (49.3%) were male and 417 (50.7%) were female. One hundred sixty-five dogs (20%) were between 6 months and 3 years of age, 387 (47%) were 4 to 6 years old, and 271 (33%) were older than 6 years of age. Three hundred sixty-three dogs (44%) were from the TM zone, 172 (21%) from the TC zone, and 288 (35%) from the DD zone.

The overall seroprevalence was 21% (172 positive dogs of 823). Of the 172 seropositive dogs, 23 (13%) were 6 months to 3 years old, 70 (41%) were between 4 to 6 years old, and 79 (46%) were older than 6 years (Chi-square 17.848,  $p < 0.001$ ). No difference in seroprevalence was found between male dogs (84/406, 21%) and female

dogs (88/417, 21%). One hundred and sixty-three dogs (95%) lived outdoors. Ninety dogs (53%) showed mild to severe symptoms of HW disease.

Table 2 shows the seroprevalence values within the different breeds: Podenco canario (45%), Dogo canario (previously named Presa canario) (34%), German shepherd (33%), large crossbreed (21%), medium crossbreed (18%), and Poodles (10%). Statistical analysis was performed only for those breeds accounting for more than 5% of the total sample. Seroprevalence values between large breeds (Padengo and Dogo canario, German shepherd and large crossbreed), medium crossbreeds and small breeds differed significantly (Chi-square 4.684,  $p < 0.05$ ).

Table 3 reports HW seroprevalence in dogs from the different climate zones. Values were 22% in both TM and DD zones (80/363 and 64/288, respectively) and 16% in dogs from TC zone (28/172) (Chi-square 2.811,  $p = 0.245$ ).

## Discussion

In the present survey, HW seroprevalence in dogs from Tenerife Island was 21%. Previous surveys have reported seroprevalences of 41.8% (Valladares et al. 1987) and 23% (Stenzemberger and Gothe 1999). Several factors can affect HW prevalence in endemic areas, not excluding owner

**Table 2** HW seroprevalence in dog breeds from Tenerife Island

Breed <sup>1</sup>			HW prevalence		Indoor/outdoor positive dogs
	No. sampled	No. positive dogs	Within breed	Within positive dogs	
Large breeds					
Podenco canario	106	48	45%	28%	0/48
Dogo canario	71	24	34%	14%	0/24
German Shepherd	64	21	33%	12%	3/18
Large crossbreed	193	41	21%	24%	1/40
Medium breed					
Crossbreed	142	26	18%	15%	2/24
Small breeds					
Poodles	43	4	10%	2%	2/2
Various breeds	204	8	4%	5%	1/9
Total	823	172	9/163		

<sup>1</sup>The statistic was performed for breeds representing more than 5% of total sample

**Table 3** HW seroprevalence in dogs from the different climate zones of Tenerife Island

Climate zones	No. sampled	No. positive dogs	HTW prevalence		Indoor/outdoor positive dogs
			Within the area	Within the positive dogs	
Temperate/mild (TM)	363	80	22%	47%	4/76
Temperate/cold (TC)	172	28	16%	16%	2/26
Dry/desert (DD)	288	64	22%	37%	3/61

compliance to chemoprophylactic treatment. In Tenerife, a commercial ivermectin formulation for canine HW prevention was first introduced in the late 1980s, but its use only became routine from the mid 1990s, mainly in companion dogs. HW preventives are usually not administered to hunting and guard dogs (Montoya and Morales, personal communication) Even though the routine use of preventives has resulted in a significant decrease of prevalence values compared to the 1987 survey (Valladares et al. 1987), no further decrease has been observed from 1999 to the present (Stenzernberger and Gothe 1999). This suggests that an active reservoir is present in the Island and it is able to maintain the HW prevalence high.

The distribution of seroprevalence by sex, age (the risk of infection increases with age), exposure (inside vs outside), and breeds was in agreement with the epidemiological findings of HW infection (Glickman et al. 1984). Larger dogs are more attractive for mosquito intermediate hosts, and HW prevalence is usually higher in larger animals than in medium and small ones, as confirmed by our results. Furthermore, Padengo, Dogo, and German shepherds are frequently used as hunting or guard dogs; most live outdoors and are, therefore, exposed to a higher risk of mosquito bites. In this survey, only 9 of 172 HW seropositive dogs lived indoors.

Climate is a critical factor in the prevalence of the disease. The rate of *D. immitis* maturation to infective third-stage larvae (L3) in the mosquito vector(s) depends mainly on the environmental temperature, and there is a threshold of about 14°C below which development will not proceed (Fortin and Slocombe 1981; Genchi et al. 2005). Though the three main Tenerife zones studied here have different climatic characteristics, these differences are apparently not enough to influence infection prevalence.

In conclusion, the data of this survey shows that canine HW disease is widely and homogeneously distributed in the canine population of Tenerife Island, and seroprevalence

has remained quite high in spite of the introduction of preventive treatment more than 10 years ago. Nevertheless, because very few dogs are introduced onto the Island, and given the limited extension of the island (2,034 km<sup>2</sup>), it could be of interest to involve practitioners in a control campaign to understand if and how many dogs should be treated to eradicate the parasite from the island.

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### **OBJETIVO 3**

Valorar las variaciones de la incidencia de *D. immitis* en perros, en la isla de Gran Canaria, entre 2000 y 2008 y determinar si el podenco canario constituye una población de riesgo.

### **ARTÍCULO**

**Epidemiological survey of canine heartworm disease on the island of Gran Canaria, (Canary Islands-Spain) between 2000-2008.**

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## Short communication

# Epidemiological survey of canine heartworm disease on the island of Gran Canaria (Canary Islands – Spain) between 2000 and 2008

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## ABSTRACT

Canine cardiopulmonary dirofilariosis caused by *Dirofilaria immitis* is endemic on the island of Gran Canaria. Epidemiological surveys have shown differences in the prevalence of heartworm disease in the canine population of the island between 1989 and 1998. The aim of the present study was to follow-up the prevalence of *D. immitis* in both the canine population as a whole and the Canarian Warren Hound population on Gran Canaria between 2000 and 2008. Prevalences observed were always significantly higher in the Canarian Warren Hound population than in the entire canine population. A significant decrease in the prevalence (from 30.19% to 19.36%) in the whole population was observed. In the Canarian Warren Hound the prevalence decreased slightly between 2000 and 2007 (40.42–34.65%), rising in 2008 to values higher than those observed at the beginning of follow-up study (41.6%). These changes are mainly attributed to lack of preventive chemotherapy and the prolonged exposure of the Canarian Warren Hound to mosquito bites. As this breed can act as a natural reservoir in Gran Canaria, regular surveillance is needed to detect changes in the epidemiological picture on this island.

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## 1. Introduction

Cardiopulmonary dirofilariosis caused by *Dirofilaria immitis* is a vector-borne disease, affecting dogs and cats living in temperate and tropical areas throughout the world (Genchi et al., 2005). It is also transmitted to humans where it can produce benign pulmonary nodules which, when X-rays are carried out, can be confused with lung cancer (Simón et al., 2009). Different species of culicid mosquitoes of the genera *Culex*, *Aedes* and *Anopheles* act as vectors for dirofilariosis (Cancrini and Gabrielli, 2007). Climatic conditions, mainly high temperatures and humidity, greatly affect the density of vector populations and, consequently, the transmission and prevalence of heartworm disease (HWD) (Genchi et al., 2009). Other factors, such as

the adequate application of prophylaxis (ivermectin in a monthly dosage) and the movement of pets from one area to another, affect the epidemiology of the disease (Simón et al., 2009).

There is much information available on the prevalence of Canine HWD throughout the world (Genchi et al., 2007). However, there are few endemic areas where surveys have been carried out in order to detect changes in epidemiological patterns of HWD in animal reservoirs. Studies carried out over intervals of several years show the spread of the disease in the canine population in Northern Italy between 1968 and 2000 (Genchi et al., 2001). The disease developed in small focal areas, spreading out over the entire Po valley and the Tuscany region, and had begun to move into the South of Switzerland (Genchi et al., 2005; Otranto et al., 2009).

Recent studies carried out in mainland Europe, United Kingdom and Argentina using technologies such as Geographic Information System (GIS) and Remote Sensing (RS)

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**Table 1**  
Number of dogs on the island of Gran Canaria with associated % for totals and each year.

Year	2000	2002	2007	2008	Total
Canarian Warren Hound	94 (18.68%)	87 (17.29%)	202 (40.15%)	120 (23.85%)	503 (27.74%)
Other breeds	161 (81.32%)	223 (82.71%)	349 (59.85%)	577 (76.15%)	1310 (72.25%)
Male	155 (60.78%)	198 (63.87%)	249 (45.19%)	352 (50.50%)	954 (50.50%)
Female	100 (39.21%)	112 (36.12%)	302 (54.8%)	345 (49.49%)	859 (49.49%)
Total canine population, n/(%)	255	310	551	697	1813

confirm the spread of HWD and predict that many areas that are currently considered non-endemic could become endemic for HWD in the near future if the present tendencies towards temperature rise continue (Genchi et al., 2005; Vezzani and Carbajo, 2006; Medlock et al., 2007).

So far, there is an incomplete picture of the distribution of canine HWD in Spain (Montoya et al., 2007). The first epidemiological survey was carried out by Valladares et al. (1987) on the island of Tenerife. Following this, Guerrero et al. (1989) completed an epidemiological study over the whole of Spain, minus 10 provinces. More recently, local surveys have been made in Alicante (Cancrini et al., 2000), La Coruña (Simón et al., 2009) and La Rioja (Morchón et al., 2010). However, no surveys have been carried out studying the changes in the prevalence of canine HWD in endemic areas in Spain except on the island of Gran Canaria where Montoya et al. (1998) observed a drastic rise in the prevalence of canine HWD in 1994 in comparison to data obtained in 1989 (Guerrero et al., 1989). This rise was then followed by a moderate decline until 1996 (Montoya et al., 1998).

The aim of this current study is to continue the analysis of changes in the prevalence of HWD on Gran Canaria between 2000 and 2008, in both the entire canine population and more specifically within the population of an autochthonous breed of dog called the Canarian Warren Hound (CWH). This breed of dog could constitute an epidemiological high risk group within that of the whole canine population on the island as a result of the handling it receives and the type of activity it is required to carry out.

## 2. Materials and methods

### 2.1. Sample and sampling procedure

One thousand and eight hundred thirteen privately owned dogs (954 males and 859 females) presented to veterinary clinics for routine health examination between 2000 and 2008 were included in the study. Five hundred and three of these dogs were CWH.

During the year 2000 blood samples were collected from 255 dogs, 49 of these dogs being CWH. In 2002, 310 blood samples were collected (87 from the CWH). In 2007, 551 blood samples were obtained (202 from the CWH) and, in 2008, 97 blood samples (120 CWH) were collected (Table 1).

The criteria for inclusion of dogs were dogs over 5 months of age, never having received treatment for heartworm disease, no previous history of heartworm infection and owner consensus to participation in the survey. A complete record was kept for each dog, including identification

(age, sex, breed), clinical history, and demographic data. The study was approved by the ethical committee of Veterinary Medicine Service of Las Palmas de Gran Canaria University and it was carried out in accordance with the current European legislation on animal protection.

### 2.2. Serology

All canine serum samples were analyzed for circulating *D. immitis* antigens in order to detect heartworm infection. They were analyzed using a commercial ELISA test kit (Canine Heartworm Antigen, IDEXX Laboratories Inc., Westbrook, Maine, USA) according to manufacturer's instructions. A sample was considered positive if it developed more color than the negative control. Each test was valid if the positive control developed a distinct blue color and the negative control was transparent or developed a very faint color.

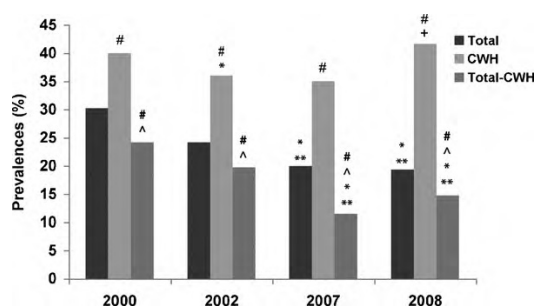
### 2.3. Statistical analysis

The data were analyzed using the SPSS Base 17.0 software for Windows. The descriptive analysis of the variables considered was carried out by studying the proportions in the qualitative variables. The Chi-square test was performed to compare proportions. In all the cases the significance level was established at  $p < 0.05$ .

## 3. Results

A clear decline in the seroprevalence of *D. immitis* can be observed in the canine population as a whole on the island of Gran Canaria (Fig. 1). Between 2000 and 2007 the seroprevalence of HWD in the total canine population of the island decreased from 30.19% to 19.36%, which tended to stabilize in 2008. There are significant differences between the seroprevalences of the disease in the canine population as a whole ( $p < 0.05$ ), over the years analyzed apart from the last two years of the study (2007 and 2008).

Changes in the prevalence of the HWD in the CWH differ from the rest of the canine population of the Gran Canaria Island. Between the years 2000 and 2007 the decline in seroprevalence was less marked within the population of the CWH than that observed in the canine population as a whole (from 40.42% to 34.65%) (Fig. 1). There are significant differences between the seroprevalences of the disease in 2000 and 2002 ( $p < 0.05$ ), whilst there are no significant differences between seroprevalences in 2002 and 2007. In 2008 the seroprevalence in the CWH population rose, once again, to values above those of the year 2000 (41.6%). There are significant differences between the seropreva-



**Fig. 1.** Canine seroprevalence of *D. immitis* on the island of Gran Canaria between 2000 and 2008. Significant differences were defined as  $p < 0.05$  between groups (in total canine population: \* and \*\* indicate  $p < 0.05$  with respect to years 2000 and 2002, respectively; in CWH: #  $p < 0.05$  with respect to year 2000, +  $p < 0.05$  with respect to year 2007, #  $p < 0.05$  with respect to the total canine population; in total canine population-CWH: \* and \*\* indicate  $p < 0.05$  with respect to years 2000 and 2002, respectively; #  $p < 0.05$  with respect to the total canine population, ^  $p < 0.05$  with respect to the total canine population-CWH).

lences of 2007 and 2008 ( $p < 0.05$ ). Over the four years of the study there are also significant differences between the seroprevalences of the infection to be found in the CWH and the entire canine population ( $p < 0.05$ ). The evolutive pattern of the whole population, without the inclusion of the CWH, is similar to that observed in CWH population alone, with a decrease in seroprevalence between 2000 and 2007 and a moderate increase in 2008.

#### 4. Discussion

Canine HWD is a vector-borne parasitosis. Therefore, changes in climatic conditions affect vector mosquito populations and in turn modify the distribution and prevalence of parasites. In addition, owner's care and handling of dogs which are both popular pets and working animals also influence the changes in epidemiological parameters of canine HWD (Genchi et al., 2005). The follow-up study of HWD in canine populations living in some endemic areas has shown that the prevalence of the infection has gone through some noticeable changes over time (Genchi et al., 2001, 2005; Otranto et al., 2009). For this reason, continued epidemiological surveillance is needed in order to inform the maintenance or modification of control measures according to variations that take place in each endemic area.

In Spain epidemiological surveys have only been carried out, albeit on a limited basis, on the island of Gran Canaria (Montoya et al., 1998). The data published establish notable changes in the seroprevalence of *D. immitis* in the canine population along with a significant rise in this prevalence from 36.7% in 1989 to 67.02% in 1994. This has been the highest prevalence of canine HWD observed in Spain, a prevalence which is similar to that reported in Cuba and Japan, where climatic conditions are similar to those of Gran Canaria. This high prevalence has been attributed to the prolonged exposure of dogs to mosquito bites, and the lack of application of preventive measures (Genchi et al., 2005).

A decline was then observed in the prevalence of HWD between 1994 and 1996. Montoya et al. (1998) attributed this to the fact that preventative therapy had begun to be administered to the canine population. These measures were mostly applied in urban areas on the island and these authors suggested that it was likely that these preventative measures were only applied to the rest of the canine population on the island, which was made up mostly of hunting dogs and other breeds of guard dogs, in very few instances.

This current research shows that during the study period the prevalence of HWD has not evolved in the same way within the canine population as a whole as in the CWH population. In the total population the prevalence of HWD decreased between 2000 and 2007, tending to stabilize in 2008. Meanwhile, in the CWH there has been a significantly smaller decrease in the prevalence between 2000 and 2007, compared with the total population, followed by a dramatic increase in 2008. The CWH is mostly used for hunting on the island of Gran Canaria. These dogs are kept in cramped, unhygienic conditions and are poorly fed. Due to the conditions in which they are kept these dogs do not receive preventative therapy and have no protection against mosquito bites. Moreover, they tend to live outdoors in rural communities, where the climatic conditions are best suited to the development of vector populations. Agriculture and livestock farming are very common activities in these areas. Average temperatures are around 18 °C and there are many ponds and reservoirs of water to be found which are ideal for mosquito development. In these areas there are also many villages with large gardens or areas of crops (Montoya et al., 1998). In these conditions the CWH living on Gran Canaria can act as a natural host reservoir for HWD.

There is a risk that if preventative measures decrease in the rest of the canine population there could be another rise in the prevalence throughout the whole canine population. Moreover, the existence of this canine host reservoir could constitute a risk factor for the development pulmonary dirofilariosis in humans. Thus it is not necessary therefore that epidemiological surveillance continue. Firstly, in order to keep up to date with changes that may occur in HWD in the canine population on Gran Canaria and secondly to provide information for medical practitioners in order that HWD may be included in a differential diagnosis for pulmonary nodules in humans.

#### Acknowledgments

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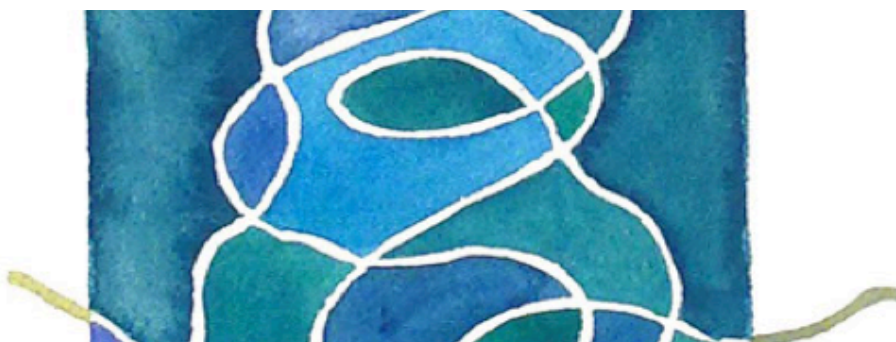
#### **OBJETIVO 4**

Conocer la seroprevalencia de *D. immitis* en la población humana y su relación con la prevalencia en perros en la isla de Gran Canaria (año 2008).

#### **ARTÍCULO**

**Canine dirofilariosis caused by *Dirofilaria immitis* is a risk factor for the human population on the island of Gran Canaria, Canary Islands, Spain.**

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# Canine dirofilariosis caused by *Dirofilaria immitis* is a risk factor for the human population on the island of Gran Canaria, Canary Islands, Spain

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**Abstract** The aim of the present study was compare the prevalence of *D. immitis* in dogs and seroprevalence in humans of Gran Canaria (Canary Islands, Spain) taking into consideration the four isoclimatic areas of the island. A close relationship between the prevalence of *Dirofilaria immitis* in dogs and the seroprevalence in humans, in each isoclimatic area, was observed. The highest seroprevalence of infection in both canine and human hosts were found in a strip of mid-range altitude with 25.47% and 30.4% in dogs and 25.66% and 29.73% in humans, respectively. The coastal zone and the highest part of the island have prevalences significantly lower. These results demonstrate that the risk of infection by *D. immitis* in the human population in each area is tied to the prevalence in the canine population. Physicians should be alerted to the possibility of finding cases of human pulmonary dirofilariosis amongst the inhabitants of the island.

## Introduction

Cardiopulmonary dirofilariosis (heartworm disease, HWD) caused by *Dirofilaria immitis* is a vector-borne disease,

affecting primarily dogs and cats from temperate and tropical areas of the world. Different species of culicid mosquitoes of the genera *Culex*, *Aedes*, and *Anopheles* act as vectors for the dirofilariosis. Some of these species feed indistinctly on animal reservoirs and man. Thus, in endemic areas, zoonotic infections through *D. immitis* can occur (Simón et al. 2009a, b).

In Europe, the highest prevalences have been reported in the canine populations of the Mediterranean countries. Moreover, heartworm disease is being detected with increasing frequency in central and Northern European countries as a consequence of the global warming and travels (Genchi et al. 2005). In Spain, the distribution of canine dirofilariosis is incompletely known because epidemiological studies have not been carried out in some provinces (Guerrero et al., 1989; Montoya et al., 2007). These studies do not reveal the existence of canine *D. immitis* infections in the Northern provinces of the Iberian Peninsula. Nevertheless, canine infections in two of these provinces have been recently reported (Morchón et al. 2009; Simón et al. 2009b), suggesting both that the lack of data is due more to the absence of studies than to the real lack of dirofilariosis and to a probable expansion of the infection from Southern endemic provinces.

The climate of Canary Islands (Spain) is very different from those of the European continent because they are off the African Atlantic coast, only 95 km from the Western Sahara. The first epidemiological studies on canine dirofilariosis in the island of Gran Canaria showed a prevalence of 36% in dogs living in the capital of the island, Las Palmas de Gran Canaria (Guerrero et al. 1989). Prevalences of 67.02%, 58.92% and 52.18% were obtained in epidemiological surveys of the whole canine population of the island (Montoya et al. 1998),

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these being the highest prevalences observed in Spain. Data supporting the existence of the other important zoonotic species (*D. repens*) in the canine population of the island of Gran Canaria have not been published until now.

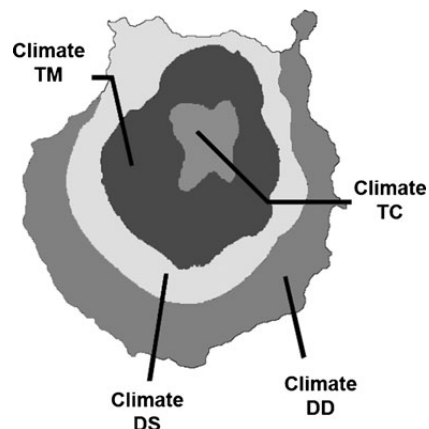
Human dirofilariasis is habitually underdiagnosed (Simón et al. 2005). The reported clinical cases reveal only a part of the human infections (Simón et al. 2009a, b) because seroepidemiological studies carried out in exposed populations show that humans contact frequently with *Dirofilaria* species (Prieto et al. 2000). Moreover, the habitual asymptomatic course of human infections can contribute to the fact that some cases pass undetected (Simón et al. 2005). In the Canary Islands, a recent study showed a high seroprevalence of specific anti-*D. immitis* IgG and IgE antibodies in the human population of the island of Tenerife (Pou-Barreto et al. 2008). Nevertheless, data regarding human dirofilariasis on the island of Gran Canaria have not been published until now, in spite of the high prevalences observed in the canine population.

In the present study, we analyze the current seroprevalence and distribution of human dirofilariasis and their relationship to the distribution of heartworm infection in the canine population of the island of Gran Canaria.

## Methods

### Physiographic and climate of the island of Gran Canaria

The island is situated near the African coast of the Sahara at 28°N, 15°O. It is circular in shape with a diameter of approximately 80 km. It has a volcanic peak of 1,949 m over the sea at its centre. This structure, its geographical location, and the constant presence of the trade winds determine the existence of four concentric isoclimatic zones (Fig. 1) These are ascending in altitude, from the coast to the highest central peak of the island (Montoya et al. 1998): (1) in the dry, desert climate zone (DD), between 0 and 200 m, there is less than 18 mm/year rainfall, temperatures are higher than 18°C, and summers are very dry. Intensive agriculture is located in this zone, mainly bananas and tropical fruits. (2) In the dry, stepparic climate zone (DS), between 200 and 500 m, the average temperature is higher than 18°C, and the rainfall is 500 mm/year. The atmosphere is cool and pleasant, and there are vines, cereal, and fruits cultivated. (3) In the temperate, mild climate zone, there are dry summers and mild winters (TM), between 500 and 1,100 m. Average temperatures ranges are from 12°C to 16°C. In winter, the weather is cold and wet, with cloud formation that benefits the soil by the way of dew and fog. Precipitation reaches 500 to 1,000 mm/year. This area of the island is characterized by green foliage and cereals and potatoes are grown here. (4) The temperate, cold climate zone (TC) has



**Fig. 1** Isoclimate zones on the island of Gran Canaria. Dry and desert climate zone (DD), dry and stepparic climate zone (DS), temperate and mild climate zone (TM), and temperate and cold climate zone (TC)

cold winters and summers with temperatures below 22°C, it lies between 1,100 and 2,000 m, it has summers with hot days and winters with cold nights; it snows some years, and precipitation is about 400 mm. Temperatures may often fall below 0°C. Most of the human (60%) and canine (44%) populations are in the DD zone, followed by the DS zone with the 20% and 22%, respectively, the TM zone with the 15% and 21%, the TC zone being the least populated area with the 5% and 13%, respectively.

### Samples analyzed

We analyzed 697 privately owned dogs (352 males and 345 females) which had been taken to veterinary clinics in 2008 for routine health examinations. The sample reflects the distribution of the canine population throughout the isoclimatic areas: 302 samples were from the DD zone, 157 from the DS, 148 from the TM, and 90 from the TC. The criteria for inclusion were >5 months of age, no HWD chemoprophylaxis, no previous history of heartworm infection, and the owner's agreement to the participation in the survey. A complete record was kept for each dog, including identification (age, sex, and breed), clinical history, and demographic data.

Serum samples from 493 humans (208 men and 285 women) taken in 2008 in a local hospital were also analyzed. By ages, 40 samples were from individuals younger than 20 years, 256 from 20 to 40 years, 142 from 40 to 60, and 55 from individuals older than 60 years. The confidentiality of the information of the patients was always maintained. Two hundred and sixty serum samples were from the DD zone, 113 from the DS, 74 from the TM, and 46 from the TC. The study was performed in accordance

with deontological regulations and the present legislation on human and animal protection.

### Procedures

The presence of circulating antigens and microfilariae of *D. immitis* were studied in the dog samples. To identify canine *D. immitis* infections, blood samples were analyzed by the Canine Heartworm Antigen, Petcheck PF IDEXX test (Westbrook, USA), for the detection of *D. immitis* circulating antigens, according to manufacturer instructions. The presence or absence of microfilariae was determined by a modified Knott (Acevedo et al. 1981).

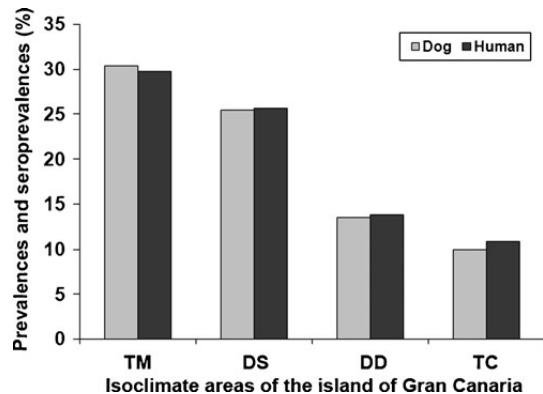
Human samples were analyzed by ELISAs to detect specific anti-*D. immitis* IgG antibodies using adult *D. immitis* somatic antigens as described previously (Simón et al. 1991). Briefly, 96-well microplates were coated with 0.8 µg of an extract of *D. immitis* adult worms. All serum samples were analyzed at a 1:100 dilution, and the secondary antibody (anti-human immunoglobulin G peroxidase-conjugated from Merck, Germany) was used at 1:4,000 dilution. Optical densities were measured at 492 nm in an Easy Reader (Bio-Rad Laboratories, Hercules, CA, USA). The cut-offs (OD=0.8) was established by calculating the mean value  $\pm 3$  standard deviations of 20 serum samples from clinically healthy blood donors living in an area free of *D. immitis*.

### Statistical analysis

The data were analyzed using the SPSS Base 17.0 software for Windows. The descriptive analysis of the variables considered was carried out studying the proportions in the qualitative variables. The chi-square test was performed to compare proportions. In all the cases, the significance level was established at  $p < 0.05$ .

### Results

One hundred and thirty-five dogs out of 697 dog samples analyzed were positive using the IDEXX test. Thus, the overall prevalence of *D. immitis* is 19.36% of which 83 (61.48%) were microfilaremic and 52 (38.52%) were amicrofilaremic infections. There are no significant differences between males (64/352, 18.18%) and females (71/345, 20.57%). Considering the isoclimatic areas, prevalences of 13.57% in DD (56.09% of microfilaremic infections), 25.47% in DS (62.50% of microfilaremic infections), 30.4% in TM (66.66% of microfilaremic infections), and 10% in TC (55.55% of microfilaremic infections) (Fig. 2) were observed. There are no significant differences between prevalences from TM and DS, nor are there among those of DD and TC, but there are



**Fig. 2** Comparative canine and human *D. immitis* seroprevalences in different isoclimatic areas on the island of Gran Canaria. Significant differences ( $p < 0.05$ ) were observed in the prevalences and seroprevalences of *D. immitis* infections in dogs and humans from TM and DS areas by one part and those observed in DD and TC areas by the other. No significant differences were observed between prevalences in dogs and seroprevalences in humans in each isoclimatic area

significant differences ( $p < 0.05$ ) between prevalences observed in TM and DS and those observed in DD and TC.

Ninety-two out of 493 human serum samples analyzed were positive in ELISA for *D. immitis*. Thus, total seroprevalence in humans is 18.66%. There are no significant differences among men (41/208, 19.71%) and women (51/285, 17.89%). When the isoclimatic areas were taken into consideration, seroprevalences of 13.85% in DD, 25.66% in DS, 29.73% in TM, and 10.86% in TC (Fig. 2) were observed. No significant differences were found between seroprevalences from TM and DS, nor were there seroprevalences between DD and TC areas. Nevertheless, as what occurs in dogs, there are significant differences ( $p < 0.05$ ) between seroprevalences of TM and DS and those observed in DD and TC. When age was considered, the highest seroprevalence was found in the youngest part of the population (<20 years), with 25%, followed by that observed in individuals between 20 and 40 years with 18.76%, and individuals between 40 and 60 years (18.7%). The lowest seroprevalence was observed in individuals older than 60 years (10.9%). There are significant differences between all the groups ( $p < 0.05$ ) except between groups of 20–40 and 40–60 years. Canine prevalences and human seroprevalences found in each isoclimatic area were very similar (Fig. 2). Statistical analysis showed no significant differences.

### Discussion

The existence of canine and feline cardiopulmonary dirofilariosis in the island of Gran Canaria is well

documented (Guerrero et al. 1989; Montoya et al. 1998; Morchón et al. 2004). Nevertheless, studies focusing on human dirofilariosis have not been carried out to date. In the present study, data demonstrating the existence of human *D. immitis* infections and their correlation with the canine prevalences are presented.

The highest *D. immitis* prevalences and the highest microfilaremic infection rates are concentrated in a strip of mid-range altitude (DS and TM areas), the prevalences being lower in the coastal strip (DD) and in the highest part of the island (TC). These differences can be attributed to the fact that in the mid-range altitude zone, there are suitable conditions of humidity and temperature for the development of the mosquito vector populations. In fact, there exist irrigated cultures and many ponds and reservoirs of water in this area. In addition, many hunting dogs with a high prevalence of HWD resident in these areas are not included in a prophylactic regime. In the other two areas, the environmental conditions limit both the vector populations and their period of activity. Moreover, the main urban areas are located in the DD zone; thus in this area, the highest human and canine population appear, chemoprophylaxis being habitually performed. Probably, the habitual practice of chemoprophylaxis in this part of the canine population is the main factor affecting the decrease of the prevalence of cardiopulmonary dirofilariosis in the island of Gran Canaria, from 52.18% in 1996 (Montoya et al. 1998) to 19.36% in the present study. Although this decrease will be analyzed in depth in the future, the present study demonstrates that the situation is not the same throughout the whole insular territory. Therefore, research considering the climatic and social situation in the different zones of the island should be carried out to obtain an accurate picture of the epidemiological situation, as this picture does not appear when the canine population as a whole is analyzed.

The seroprevalences observed in human populations living in each area show a high correlation with canine prevalences and with microfilaremic infection rates. This demonstrates, in the first place, that the existence of canine heartworm infections on the island of Gran Canaria is a potential risk for the resident human population, as has been observed in the nearby island of Tenerife (Pou-Barreto et al. 2008) and in other endemic European areas (Prieto et al. 2000; Simón et al. 1991). It is necessary to stress that the positive serology in humans only indicates a contact with the parasite (Pou-Barreto et al. 2008). Considering the high seroprevalence, the incidence of pulmonary nodules caused by *D. immitis* should be studied in the human population of the island of Gran Canaria to determine the real risk of the development of pulmonary dirofilariosis. This would enable physicians to be alerted the need for inclusion of the pulmonary dirofilariosis in the differential diagnosis of pulmonary nodules. An interesting fact is that the distribu-

tion of the human seroprevalences by age is different on the island of Gran Canaria than in other endemic areas previously analyzed. The highest seroprevalence of anti-*D. immitis* IgGs is usually detected in the oldest half of the exposed populations (Prieto et al. 2000); but in this study, the highest seroprevalence appears in the youngest part of the population. This seems to indicate that on the island of Gran Canaria, the exposure to the parasite is very early, probably due to the limited mobility of the resident individuals. Moreover, the species of vectors implicated in the transmission and their abundance and activity are other important factors. In fact, we have previously observed *D. immitis* DNA in *Culex theileri*, a strong anthropophilic species, captured in the island of Gran Canaria (own non-published data).

In conclusion, the data presented here demonstrate the existence of a high correlation between the prevalences of *D. immitis* in canine and human populations in the different isoclimatic areas of the island of Gran Canaria, confirming the risk of contact for people living in areas where there is canine dirofilariosis and the importance of climatic characteristics. Periodic epidemiological studies are necessary to determine changes in the prevalence of canine dirofilariosis and to evaluate the effectiveness of the preventive measures in each specific area. The existence of a high seroprevalence in humans should alert physicians to the possibility of finding pulmonary nodules caused by *D. immitis* among the human population of the island of Gran Canaria.

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## **OBJETIVO 5**

Estudiar la incidencia de la zoonosis en perros, gatos y humanos en las diferentes zonas isoclimáticas de Gran Canaria, y evaluar la relación entre las prevalencias de las diferentes especies (año 2010).

## **ARTÍCULO**

**Current prevalence of *Dirofilaria immitis* in dogs, cats and humans from the island of Gran Canaria, Spain**

*Veterinary Parasitology*, 176: 291-294, 2011







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# Veterinary Parasitology

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## Current prevalence of *Dirofilaria immitis* in dogs, cats and humans from the island of Gran Canaria, Spain

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### ABSTRACT

The island of Gran Canaria is a hyperendemic area for canine dirofilariasis. The aim of the present study was to provide data on *Dirofilaria immitis* in dogs, cats, and humans on this island in 2010. The data confirms the prevalence in the overall canine population (19%), with a considerably higher prevalence (43%) in the autochthonous breed of Canarian Warren hound. The prevalence in the feline population (33%) is higher than that of the canine population, and the existence of specific *D. immitis* antibodies in the inhabitants of the island of Gran Canaria (12%) is confirmed. In both cats and humans, the prevalence, according to the different climate areas on the island, is related to the prevalence of *D. immitis* in dogs in the same area, which shows the key epidemiological role played by the canine host with regard to the transmission to other hosts of *D. immitis*.

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### 1. Introduction

Cardiopulmonary dirofilariasis (heartworm disease) caused by *Dirofilaria immitis*, is a vector-borne transmitted disease, distributed worldwide, primarily affecting dogs and cats (Genchi et al., 2007). Different species of culicid mosquitoes belonging to the genera *Culex*, *Aedes*, and *Anopheles*, among others, have been implicated in the transmission of this parasite (Cancrini and Gabrielli, 2007). Because some of these mosquito species feed indiscriminately on animal reservoirs and humans, *D. immitis* can be transmitted to humans residing in endemic areas, causing benign pulmonary nodules that are frequently confused with pulmonary carcinomas (Simón et al., 2009). In Europe, dirofilariasis in animals and humans is endemic in the Mediterranean countries (Genchi et al., 2005; Simón et al., 2005). Dirofilariasis is being detected more and more frequently in central and eastern areas of the Euro-

pean continent (Simón et al., 2009). Although the Canary Islands belong politically to the European Union, they have a semitropical climate, which is influenced by the Atlantic trade winds and their proximity to the Western Sahara coast. Dirofilariasis is hyperendemic on these islands, with prevalences of 20% to 34% observed in dogs on the island of Tenerife (Valladares et al., 1987; Guerrero et al., 1989; Montoya-Alonso et al., 2006). On the island of Gran Canaria, a decrease in prevalence from 67% in 1994 to 19% in 2008 was detected in the canine population as a whole, which was attributed to the use of preventatives (Montoya-Alonso et al., 1998, 2010). In the Canarian Warren hound, a decrease in prevalence was observed between the years 2000 and 2007 (40–35%). This decrease was followed by a major increase in the year 2008 (42%) (Montoya-Alonso et al., 2010). The Canarian Warren hound is an autochthonous breed used for hunting and rat-catching in rural areas and usually does not receive preventatives.

The existence of *D. immitis* in cats on the island of Gran Canaria and in humans on the island of Tenerife has also been reported (Pou-Barreto et al., 2008). To continue the

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monitoring of dirofilariasis on the island of Gran Canaria, the current study provides data on the prevalence in 2010 and also adds original data on the prevalence in cats and humans on the island.

## 2. Materials and methods

### 2.1. Characteristics of Gran Canaria

Gran Canaria is a circular island, 40 km in diameter, situated near the African coast of the Sahara at 28°N, 15°W. The island has a truncated cone shape, and the highest central peak of the island is 2000 m high. A significant change in climate, vegetation, and mountain geography can be observed by travelling only a few kilometers. Four different isoclimatic zones are found on Gran Canaria, ascending in altitude from the coast to the central peak of the island (Rodríguez-Ponce et al., 1995; Molina et al., 1997; Montoya-Alonso et al., 1998): dry and desert zone (DD), dry and steppe zone (DS), temperate mild zone (TM), and temperate cold zone (TC).

In the DD, which runs from 0 to 200 m, there is less than 18 mm/year (0.7 in.) rainfall, temperatures are higher than 18 °C (64.4 °F), and summers are very dry. Intensive agriculture, mainly bananas and tropical fruits, is located in this zone. In the DS, between 200 and 500 m, the average temperature is higher than in the DD and the rainfall is 500 mm/year (19.7 in.). The atmosphere is cool and pleasant; vines, cereal, and fruits are cultivated. In the TM, between 500 and 1100 m, there are dry summers and mild winters. Average temperatures range from 12 °C to 16 °C (53.6–60.8 °F). In winter, the weather is cold and wet, with cloud formation that benefits the soil by the way of dew and fog. Precipitation reaches 500–1000 mm/year. This area of the island is characterized by green foliage. Cereals and potatoes are grown here. In the TC, which lies between 1100–2000 m, winters are cold and summer temperatures are below 22 °C (71.6 °F). Summer days are hot and winter nights are cold; it snows some years and precipitation is about 400 mm (15.7 in.). There is great temperature variation between day and night. Temperatures may often fall below 0 °C (32 °F).

### 2.2. Samples

Five hundred forty-seven client-owned dogs (208 were 1–3 years of age, 142 from 3 to 6 years of age, and 197 older than 6 years) presented to veterinary clinics for routine health examination in 2010 were included in the study. Sixty of these dogs were Canarian Warren hounds. One hundred nine client-owned cats (39 were 1–3 years of age, 40 were >3–6 years of age, and 30 older than 6 years) presented to veterinary clinics for routine health examination in the same year also were included. The sample reflects the distribution of the canine and feline population throughout the four different isoclimatic areas of the island of Gran Canaria (Table 1). The criteria for inclusion of dogs and cats were to be over 6 months of age, never having received treatment for heartworm disease, no previous history of heartworm infection, and owner consensus to participate in the survey. A complete

**Table 1**

Number (%) of dogs, cats, and humans evaluated on the island of Gran Canaria.

	Dogs	Cats	Humans
Population evaluated	547	109	100
Gender			
Male	267 (48.8)	56 (51.4)	35 (35)
Female	280 (51.2)	53 (48.6)	65 (65)
Climate zone			
TM	112 (20.5)	20 (18.3)	12 (12)
DS	102 (18.6)	32 (29.4)	14 (14)
DD	275 (50.3)	47 (43.1)	66 (66)
TC	58 (10.6)	10 (9.27)	8 (8)

DD, dry and desert; DS, dry and steppe; TM, temperate mild; TC, temperate cold.

record was kept for each animal, including identification (age, sex, and breed), clinical history, and demographic data.

One hundred random serum samples collected from humans (7 individuals less than 20 years of age, 84 individuals 20–60 years of age, and 9 older than 60 years) in 2010 in a local hospital were analyzed. The confidentiality of the information of the patients was always maintained, and all patients provided written consent to participate in the study. The sample reflects the distribution of the human population throughout the four different isoclimatic areas (Table 1).

The study was approved by the ethical committee of Veterinary Medicine Service of Las Palmas de Gran Canaria University and was carried out in accordance with the current European legislation on animal protection.

### 2.3. Study measurements

All canine serum samples were analyzed for circulating *D. immitis* antigens using a commercial ELISA test kit (PetChek1 HW PF, IDEXX Laboratories Inc., Westbrook, Maine, USA) according to manufacturer instructions.

Feline *D. immitis* infections were identified using serological techniques for anti-*D. immitis* and anti-*Wolbachia* (WSP) antibody detection (Morcón et al., 2004), with some modifications. In brief, the plates were coated with 0.8 µg of both antigens. Serum samples were prepared at 1/100 for anti-*D. immitis* serum antibodies and 1/40 for anti-WSP antibody detection; anti-cat IgG antibody, horseradish peroxidase-labeled (Kirkegaard and Perry Laboratories, Gaithersburg, MD) was applied at 1/4000 dilution. The optical densities (ODs) were measured in an Easy-Reader (Bio-Rad Laboratories, Hercules, CA, USA) at 492 nm. Cut-off points (ELISA *D. immitis* 0.8 and ELISA WSP 0.6) were obtained as arithmetic mean O.D. ± 3 standard deviations of sera of clinically healthy cats.

Human samples were analyzed with an ELISA test to detect the specific IgG antibody response using adult *D. immitis* somatic antigens, as described by Kartashev et al. (2011) with some modifications. Briefly, 96-well microplates were coated with 0.8 µg of an extract of *D. immitis* adult worms. All serum samples were analyzed at a 1:100 dilution and anti-human IgG (Sigma–Aldrich) was used at 1:4000 dilution. Optical densities were measured at 492 nm in an Easy Reader (Bio-Rad Laboratories). The



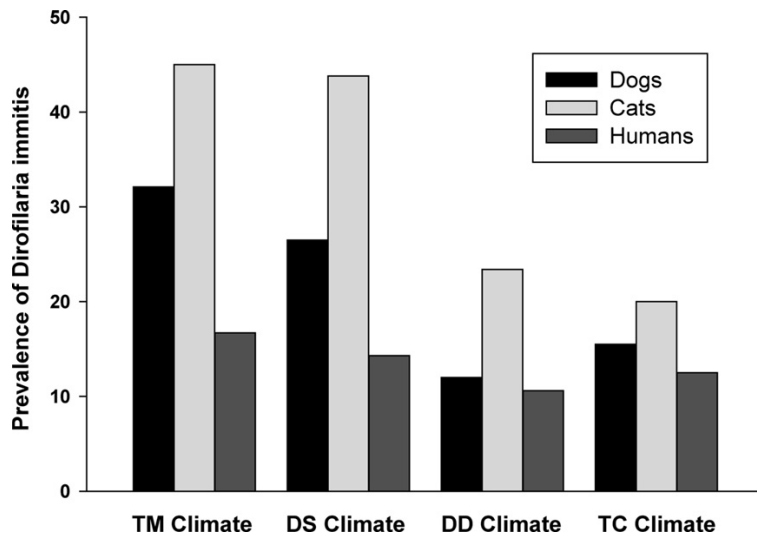


Fig. 1. Comparative canine, feline, and human *D. immitis* seroprevalences in different isoclimatic areas in the island of Gran Canaria.

cut-off (O.D.=0.8) was established calculating the mean value  $\pm 3$  standard deviations of 20 serum samples from clinically healthy blood donors living in a *D. immitis*-free area.

#### 2.4. Statistical analysis

The data were analyzed using the SPSS Base 18.0 software for Windows. The descriptive analysis of the variables considered was carried out studying the proportions in the qualitative variables. The Chi-square test was performed to compare proportions. In all the cases, the significance level was established at  $P < 0.05$ .

### 3. Results

The prevalence of *D. immitis* in the canine population of the island Gran Canaria was 19.2%. There were significant differences ( $P < 0.05$ ) in the prevalence between males (42/267, 15.7%) and females (63/280, 22.5%). Considering the isoclimatic areas, prevalences of 12.0% in the DD climate zone, 26.5% in the DS climate zone, 32.1% in the TM climate zone and 15.5% in the TC climate zone were observed. There were no significant differences between the prevalences in the TM and DS climate zones, nor were there differences between the DD and TC climate zones; however, the prevalences were significantly higher ( $P < 0.05$ ) in the TM and DS climate zones than in DD and TC climate zones. The prevalence among the Canarian Warren hound population was 43.3%. Considering the canine population, excluding the Canarian Warren hound, the prevalence was 16.2%. By age, the highest prevalence was found in the 3–6 year-old age group (35.2%; 50/142), followed by that in the >6 years age group (17.8%; 35/197); the lowest prevalence was observed in the 1–3 years age group (9.6%; 20/208). There were significant differences among the three groups ( $P < 0.05$ ).

Total seroprevalence in cats was 33.0%. There was no significant difference in the prevalence between males (32.1%; 18/56); and females (34.0%; 18/53). Prevalences of 23.4% were observed in the DD climate zone, 43.8% in the DS climate zone, 45% in the TM climate zone, and 20% in TC climate zone. There were no significant differences between prevalences in TM and DS climate zones, nor were there differences between the DD and TC climate zones, but there were significant differences ( $P < 0.05$ ) between prevalences observed in TM and DS climate zones and those observed in DD and TC climate zones. The highest prevalence was found in cats of the >6 years age group (46.8%; 14/30), followed by that in the 3–6 years age group (35%; 14/40); the lowest prevalence was observed in the 1–3 years age group, 20.5% (8/39). There were significant differences among the three groups ( $P < 0.05$ ).

Total seroprevalence in humans was 12%. There were significant differences ( $P < 0.05$ ) between men (17.1%; 6/35) and women (9.2%; 6/65). Seroprevalence was 10.6% in the DD zone, 14.3% in DS zone, 16.7% in TM zone, and 12.5% in TC zone. No significant differences were found between seroprevalences in the TM and DS climate zones, nor were there between the DD and TC climate zones. Nevertheless, as was observed in the dog population, there were significant differences ( $P < 0.05$ ) between seroprevalences of TM and DS climate zones and those observed in DD and TC climate zones. The highest seroprevalence was found in the oldest section of the population (>60 years) (22.2%; 2/9), followed by that in individuals 20–60 years (11.9%; 10/84). No anti-*D. immitis* antibodies were found in individuals <20 years of age. There were significant differences among the three age groups ( $P < 0.05$ ).

Overall, the highest prevalences of *D. immitis* in dogs, cats, and humans were found in the TM zone, followed by the DS climate zone. The lowest prevalences were found in the DD and TC zones (Fig. 1).

#### 4. Discussion

The Canary Islands is a hyperendemic area for dirofilariasis (Montoya-Alonso et al., 2007). Its geographical location and mountain geography have given rise to several areas that are isoclimatic in altitude, which in turn, determine the variations in the prevalence of *D. immitis* in the canine population (Montoya-Alonso et al., 2010). Furthermore, variations over time in the prevalence of dirofilariasis have been observed, with a gradual decrease in these in the canine population as a whole between 1994 and 2008 (Montoya-Alonso et al., 1998, 2010). Meanwhile, in the autochthonous breed of Canarian Warren hound, there was a minor decrease in prevalence between 2000 and 2007, followed by a marked increase in 2008 (Montoya-Alonso et al., 1998, 2010); a slight increase from 2008 (41.6%) was observed in this canine population in the present study (43.3%). Differences in the evolution of prevalence within the canine population have been attributed to the difference in the handling of dogs kept as pets, including the application of heartworm preventatives, and the handling of the Canarian Warren hound, which generally do not receive heartworm preventatives.

Data collected in the current study, confirms the variations in prevalences in the canine population among the different areas of the island and among different age groups. These data also establish the current prevalence in the population as a whole (19.4% in 2008 and 19.2% in 2010) for comparisons with data from previous surveys.

This study also includes data on prevalence of *D. immitis* in feline and human populations. The existence of feline heartworm infection on the island of Gran Canaria was demonstrated by Morchón et al. (2004) using serology for anti-*D. immitis* and anti-*Wolbachia* antibodies. As far as we know, this is the first time that a higher prevalence in cats over dogs has been described in the same endemic area (Genchi et al., 2007). This may be because cats on the island of Gran Canaria are not treated with preventatives, leading to the spread of this infection in this host.

In this study, specific anti-*D. immitis* antibodies have been identified in the human inhabitants of Gran Canaria. This demonstrates the risk of infection from *D. immitis* for those residing on the island, as is the case in other endemic areas (Simón et al., 2009). In both the feline and human hosts, the level of infection within the different isoclimatic areas is related to the prevalence in dogs, which shows the key epidemiological role played by the canine host as it relates to other *D. immitis* hosts.

In conclusion, the data presented confirm findings previously published and complete the epidemiological picture of dirofilariasis on the island of Gran Canaria. Moreover, it clearly shows that the handling of animals and climatic characteristics are key factors in determining prevalence in both the animal reservoirs and those people living in endemic areas.

#### Conflict of interest

The authors declare no conflicts of interest.

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## **OBJETIVO 6**

Determinar la incidencia de la dirofilariosis cardiopulmonar en pequeños animales en todo el archipiélago Canario (años 2014-15).

## **ARTÍCULO**

**Current epidemiological status of *Dirofilaria immitis* in the pet population of the Canary Islands.**

*Veterinary Parasitology, 2015-16 (submitted)*





## CURRENT EPIDEMIOLOGICAL STATUS OF *DIROFILARIA IMMITIS* IN THE PET POPULATION OF THE CANARY ISLANDS

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### ABSTRACT

Cardiopulmonary dirofilariosis (heartworm) is a zoonotic vector borne disease caused by *Dirofilaria immitis* which affects domestic dogs and cats. Two of the seven Canary Islands are historically hyperendemic areas of dirofilariosis, although no epidemiological study has ever been done including the other islands. The aim of the study was to complete the epidemiological status of cardiopulmonary dirofilariosis in the canine and feline population including all the Canary Islands. 1643 client-owned dogs and 707 client-owned cats were tested for *D.immitis* antigens (dogs), and anti-*D.immitis* and anti-*Wolbachia* antibodies (cats). The prevalence of canine dirofilariosis on the Canary Islands was 15.7%, and the seroprevalence of feline dirofilariosis was 18.1%. A remarkable disparity was found evaluating the results by island separately, from 0% in Lanzarote and El Hierro and low prevalences and seroprevalences in Fuerteventura (1.8% and 2.5% in dogs and cats, respectively), to the other 4 islands which ranged from 15.7% (dogs) and 14.3% (cats) in La Palma to 22.5% (dogs) and 24.1% (cats) in Tenerife. In addition, prevalences and seroprevalences were very variable inside each island, being these differences associated to local climate conditions. The distribution and prevalence of dirofilariosis in the Canary Islands is heterogeneous and related to the climate, demographic factors and management of the pets in the studied areas. Dirofilariosis remains hyperendemic in 4 of the 7 islands. Since *D.immitis* is a zoonosis, veterinary and sanitary authorities should be aware of the current prevalence and seroprevalence of animal dirofilariosis. The results show the need of awareness campaigns for the implementation of prophylactic measures in pets, in order to achieve a decrease in the prevalence of animal dirofilariosis in the Canary Islands.

### KEYWORDS

Animal heartworm, *Dirofilaria immitis*, prevalence, epidemiology, Canary Islands

### INTRODUCTION

Cardiopulmonary dirofilariosis (heartworm) is caused by *Dirofilaria immitis*. It is a vector-borne disease which mainly affects domestic dogs and cats, although it has also been described in other pets, like ferrets, and in wild carnivores. It is transmitted by different species of culicid mosquitoes of the genera *Culex*, *Aedes* and *Anopheles*; therefore, the prevalence of the infection is influenced by the climatic conditions: those regions with high temperatures and humidity benefit the perpetuation and reproduction of the mosquito vectors and, hence, the transmission (Carretón et al., 2012; Simón et al., 2012).

Canine dirofilariosis constitutes a risk for the human population since *D.immitis* can also be transmitted to humans, in which produce benign pulmonary nodules; these are often confused with lung cancer in X-ray and thoracic CT scans (Simón et al., 2009).

Europe currently presents high prevalences of canine dirofilariosis in the southern Mediterranean countries; in addition, it has been reported a spreading of the disease towards non-endemic areas and countries previously considered free of dirofilariosis (Genchi et al., 2009; Morchón et al., 2012).

Although the Canary Islands belong politically to the European Union, they are located near the Western Sahara coast. Dirofilariosis is hyperendemic in the two most populated islands, showing prevalences from 41.8% in 1987 to 21% in 2003 in dogs from Tenerife (Valladares et al., 1987; Guerrero et al., 1989; Stenzernberger and Gothe, 1999; Montoya et al., 2006) and a decrease from 67% in 1994 to 19% in 2011 in dogs from Gran Canaria (Montoya et al., 1998; Montoya-Alonso et al., 2010; 2011). With regard to the cats, a seroprevalence of 33% was found in Gran Canaria in 2011, being the first time that a higher seroprevalence in cats over prevalence in dogs has been described in the same endemic area. Since the study detected antibodies and, therefore, exposure to *D.immitis*, demonstrated that the feline population was at high risk of infection (Montoya-Alonso et al., 2011).

A geo-environmental model for the prediction of potential transmission risk of *Dirofilaria* in Spain predicted the highest number of generations in the SouthWestern quadrant of the Iberian Peninsula, Mediterranean regions and the Canary and Balearic islands; furthermore, the longest period of transmission of *Dirofilaria* appeared in the Canary Islands where it occurs throughout the year (Simón, 2014). Despite this worrying evidence, no epidemiological study including all the Canary Islands has been ever done. For this reason, the aim of the present study was to complete the epidemiological status of cardiopulmonary dirofilariosis in the canine and feline population of the Canary Islands.

## METHODS

### **1. Location and climate of the Canary Islands**

The Canary Islands are situated 97 kilometres (60 miles) from the African coast of the Sahara, between the meridians 13° and 19° W and the parallels 27° and 30° N. The Archipelago consists of seven islands, all of which are volcanic in origin. The islands are from largest to smallest: Tenerife, Fuerteventura, Gran Canaria, Lanzarote, La Palma, La Gomera and El Hierro. Further demographic and geographic data are included in table 1.

Overall, the climate is subtropical and desert, moderated by the sea and in summer by the trade winds. According to the position of the islands with respect to the north-east trade winds and to the altitude of each island, the climate varies from mild and wet to very dry. Indeed, different climates can be found by travelling only a few kilometres in each island, by ascending in altitude from the coast to the central peak. According to the Köppen Climate Classification (Production Department of the State Meteorological Agency of Spain, 2012), the Canary Islands present hot desert (BWh) and cold desert (BWk) climates, characterized because the evaporation exceeds precipitation on average but is less than half potential evaporation, and average temperature is >18°C (BWh) and <18°C (BWk). The hot steppe (BSh) and cold steppe (BSk) climates are characterized because the evaporation exceeds precipitation on average but is less than potential evaporation and the difference between these two measures is less than what is found in a BW climate. Average temperature is >18°C (BSh) and <18°C (BSk). The temperate with hot and dry summer (Csa) climate is characterized by having hot summers with the average temperature in the warmest month above 22°C. The temperate with dry and warm summers (Csb) climate is characterized by having warm summers with the average tem-



perature in the hottest month below or equal to 22°C, and with 4 months or more with the average temperatures above 10°C. The areas in which the described climates are present in each island can be observed in figure 1.

## **2. Sample collection**

1643 client-owned dogs, presented to veterinary clinics for routine health examination between May 2014 and February 2015, were included in the study. Of these, 884 were female and 759 were male; 1209 were pure-bred dogs and 434 were mixed-bred dogs.

707 client-owned cats, presented to veterinary clinics for routine health examination in the same interval of date, were included. Of these, 368 were female and 339 were male; 159 were pure-bred cats and 548 were mixed-bred cats.

The criteria for inclusion of dogs and cats were being over 6 months of age, never having received treatment for heartworm disease, no previous history of heartworm infection, and owner consensus to participate in the survey. A complete record was kept for each animal, including identification by age, sex and breed; clinical history, and demographic data. The distribution by islands of dogs and cats included in the study are enumerated in table 1.

The study was approved by the ethical committee of Veterinary Medicine Service of Las Palmas de Gran Canaria University and was carried out in accordance with the current European legislation on animal protection.

## **3. Laboratory analysis**

Blood samples were collected from the cephalic or jugular vein, placed in 3 ml serum tubes and centrifuged. Serum was kept at -20°C until tests were performed.

All canine serum samples were analysed for circulating *D. immitis* antigens using a commercial immunochromatographic test kit (Urano test *Dirofilaria*®, Urano Vet SL, Barcelona, Spain) according to manufacturer's instructions.

Feline *D. immitis* infection was identified using serological techniques for anti-*D. immitis* and anti-*Wolbachia* antibody detection (Morchón et al., 2004) with some modifications. In brief, the plates were coated with 0.8 mg of *D. immitis* somatic antigen and *Wolbachia* surface protein (WSP). Serum samples were prepared at 1/100 for anti-*D. immitis* serum antibodies and 1/40 for anti-WSP antibody detection. Horseradish peroxidase-labelled anti-feline IgG antibody (Kirkegaard and Perry Laboratories, Gaithersburg, Maryland, USA), was applied at 1/4000 dilution. The optical densities were measured in an Easy-Reader (Bio-Rad Laboratories, Hercules, California, USA) at 492 nm. Cut-off points of enzyme-linked immunosorbent assay (ELISA) *D. immitis* 0.8 and ELISA WSP 0.6 were obtained as arithmetic mean optical density  $\pm 3$  standard deviations of sera of clinically healthy cats. Seropositivity was considered when animals were positive to both tests, as registered by previous epidemiological studies (Montoya-Alonso et al., 2011; 2015; Vieira et al., 2015).

## **4. Statistical analysis**

Data were analysed using SPSS Base 20.0 software for Windows (SPSS Inc./IBM, Chicago, Illinois, USA). Descriptive analysis of the considered variables was carried out considering the proportions of the qualitative variables. We performed chi-square and Fischer exact tests to compare proportions. In all cases, the significance level was established at  $P < 0.05$ .

## RESULTS

The prevalence of canine dirofilariosis on the Canary Islands was 15.7%, with a greater prevalence of 17.1% in females compared with 14.1% in males ( $p < 0.5$ ). By age, prevalence was 16.4% for dogs  $< 3$  years, 17.1% for dogs aged 3–6 years and 12.3% for those  $> 6$  years. There were no statistically significant differences among the three groups. The results obtained by islands are illustrated in figure 2.

The mean age of the infected dogs was 4.6 years, being found infected dogs from 9 months to 16 years old. By breeds, 18.5% of pure-bred dogs were infected vs 7.8% of mixed-bred dogs ( $p < 0.05$ ). However, when the Canary Hound was analysed separately from the rest of the breeds, a prevalence of 30.7% was obtained compared to 9.3% in the rest of the studied dogs. There were statistically significant differences between the prevalences found in the Canary Hound and the total canine prevalence ( $p < 0.05$ ) and between the Canary Hound and the rest of the studied breeds ( $p < 0.05$ ). Prevalences of Canary Hound compared with the other dogs by islands are shown in figure 3.

When cats were evaluated, the seroprevalence in the Canary Islands was 18.1%. There were no statistically significant differences in the prevalence between males (18.3%) and females (17.9%). By age, seroprevalence was 20.1% for cats  $< 3$  years, 16.8% for cats aged 3–6 years and 14.6% for those  $> 6$  years. There were significant differences among the three groups ( $p < 0.05$ ). The mean age of the seropositive cats was 3.6 years, being found seropositive cats from 6 months to 19 years old. By breeds, 20.2% of the seropositive cats were mixed-bred compared to 7.4% of pure-bred cats seropositive to *D. immitis* ( $p < 0.05$ ). The results obtained by islands are illustrated in figure 2.

When climates were evaluated separately, the highest prevalences were found in the Csb climate (23.8% and 25.5% in dogs and cats, respectively). In dogs, prevalence was followed by the BS climate (21%) and the Csa climate (19.5%). In cats, the Csa climate presented the next highest prevalence (22.1%) followed by the BS climate (16.7%). There were no statistically significant differences between the canine and feline prevalences in the BS, Csa and Csb climate zones.

In both dogs and cats, the lowest prevalences were found in the BW climate (2% and 13.8%, respectively). There were statistically significant differences between the BW climate and all the other described climates ( $p < 0.05$ ). The results found in each climate by islands are described in table 2.

## DISCUSSION

Epidemiological studies on dirofilariosis in the Canary Islands have been conducted to the present exclusively in two islands, Gran Canaria and Tenerife (Valladares et al., 1987; Guerrero et al., 1989; Montoya et al., 1998; Stenzernberger and Gothe, 1999; Montoya et al., 2006; Montoya-Alonso et al., 2010; 2011). The present epidemiological study includes the 7 islands of the Archipelago, which reveals a remarkable disparity in the prevalences between islands and also between different areas within a same island. These differences can be attributed to the climatic singularities present in each island and to demographic factors (Montoya-Alonso et al., 2011; Production Department of the State Meteorological Agency of Spain, 2012). According to the data obtained in the present study, canine and feline dirofilariosis are absent or present with very low prevalences where the desert climate predominates, like in the easternmost Canary Islands Fuerteventura and Lanzarote.

Dirofilariosis was absent in El Hierro, despite its climatic conditions which are favourable for the transmission. This situation is attributed to the low population density of the island and the unusual movement of animals from other islands, which prevents from the introduction of reservoir dogs.

The rest of the islands presented moderate to high prevalences of *D. immitis*, both in dogs and cats, predominantly in areas with steparic and temperate climates, where moisture is provided by rainfall, influence of humid trade winds and/or existence of open ponds for water storage (Simón et al., 2014). Moreover, the coastal areas present optimal climatic conditions for heartworm transmission, in addition to higher concentrations of inhabitants, since the biggest cities and towns of the islands are located there (Montoya et al., 2006; Montoya-Alonso et al., 2011).

In the islands where canine dirofilariosis was present, the Canary Hound presented prevalences significantly greater, compared to the other breeds. Similar results were described in previous studies in Gran Canaria and Tenerife, being the Canary Hound considered the most important natural reservoir for *D. immitis*, mostly because it is kept outdoors in rural areas in unhygienic conditions exposed to mosquito bites and generally do not receive preventative therapy (Montoya et al., 2006; Montoya-Alonso et al., 2010; 2011). By age, the highest prevalence was found in dogs from 3 to 6 years, similar to the results from other studies (Montoya-Alonso et al., 2011).

It can be considered that the current canine epidemiological situation is more or less stable with a slightly growing trend in Gran Canaria and Tenerife: previous studies showed prevalences of 20% in 1989, 22.6% in 1999 and 21% in 2003 in Tenerife, which not differ much from the current prevalence of 22.5% (Guerrero et al., 1989; Stenzernberger and Gothe, 1999; Montoya et al., 2006). Regarding Gran Canaria, since 1994 canine dirofilariosis has declined from 67.02%, to 30.19% in 2000, 19.36% in 2008 and 19.2% in 2011 (Montoya et al., 1998; Montoya-Alonso et al., 2010; 2011) while the current result of 20.7% breaks the downward trend. Furthermore, compared to a previous study (Montoya-Alonso et al., 2011), there is a fall of the prevalence from 12% to 3.42% in the urban areas, while a high increase from 32.1% to 40% is observed in rural communities and crop areas of Gran Canaria. The most important factor influencing these changes could be the current economic crisis and the differences in the economic level of each population, since many families are terminating the veterinary services and interrupting the administration of chemoprophylaxis or giving concentrated ivermectin formulas not approved for heartworm prevention (Montoya-Alonso and Carretón, personal communication). Because the lack of previous data in La Palma and La Gomera, we cannot discuss possible changes in the distribution and prevalence of canine dirofilariosis in those islands.

As in dogs, this is the first complete epidemiological study of *D. immitis* in cats in the Canary Islands. Only previous studies have demonstrated the existence of feline seropositivity in Gran Canaria using similar serological techniques as described in this study (Morchón et al., 2004; Montoya-Alonso et al., 2011). Feline heartworm disease has spread and increased during the past few years throughout the world (Simón et al., 2012). However, unlike in dogs, the diagnosis of feline dirofilariosis presents a challenge, mainly because cats usually show a low parasite burden, frequently leading to a negative antigen test. Since the present study was designed to detect antibodies of anti-*D. immitis* in feline serum samples, the results demonstrate exposure to the parasite rather than necessarily active infection and further research is required to confirm the infection with adult parasites (Litster and Atwell, 2008). Cats living in the Canary Islands showed an important rate of exposure to *D. immitis* in those islands where canine dirofilariosis is present and, consequently, a high risk of developing heartworm disease.

For the first time, a complete epidemiological study of canine and feline dirofilariosis in the Canary Archipelago is conducted, revealing that the distribution and prevalence of the infection is related

to the different climates present in the islands, demographic factors and human factors related to the management of the pets. Consequently, 4 of the 7 islands are hyperendemic while dirofilariosis is absent or present a low prevalence in the other 3 islands; therefore, it is not appropriate to refer to the Canary Islands as a single hyperendemic area.

The Canary Hound presents significantly higher prevalences than the other breeds, being a dangerous reservoir of the disease for both other pets and humans, considering the zoonotic aspect of the infection (Montoya-Alonso et al., 2010b). Consequently, veterinarians, physicians and owners of pets should be alerted about the need for an adequate management of dirofilariosis, including the widespread use of diagnostic tools and prophylactic measures, in order to achieve a decrease in the prevalence of animal dirofilariosis in those Canary Islands where heartworm infection is present.

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## TABLES

Island	Area (Km2) *	Inhabitants **	Density** (inhabitants/km2)	Studied Dogs	Studied Cats
Tenerifé	2.034	889.936	437	409	166
Gran Canaria	1.560	851.157	546	478	338
La Gomera	370	20.721	56	98	36
La Palma	708	83.456	118	305	56
El Hierro	269	10.675	40	74	17
Fuerteventura	1.660	106.930	64	162	40
Lanzarote	846	141.940	168	117	54
Total	7.447	2.104.815	283	1643	707

**Table 1.** Distribution of the studied animals by islands and additional demographic data of the Canary Islands. Source: Instituto Canario de Estadística (ISTAC) [www.gobiernodecanarias.org/istac](http://www.gobiernodecanarias.org/istac), data from (\*) Instituto Geográfico Nacional (\*\*) Instituto Nacional de Estadística (INE).

DOGS	Tenerife	Gran Canaria	La Gomera	La Palma	El Hierro	Fuerteventura	Lanzarote
BW	8.3% (1/12)	3.4% (6/175)	0% (0/23)	--	0% (0/6)	1.8% (3/162)	0% (0/117)
BS	26.5% (39/147)	25.6% (43/168)	20.9% (9/43)	15.2% (7/46)	0% (0/20)	--	--
CSa	16% (12/75)	40% (26/65)	43.7% (7/16)	10.6% (16/151)	0% (0/6)	--	--
CSb	28% (49/175)	34.3% (24/70)	0% (0/16)	23.1% (25/108)	0% (0/41)	--	--

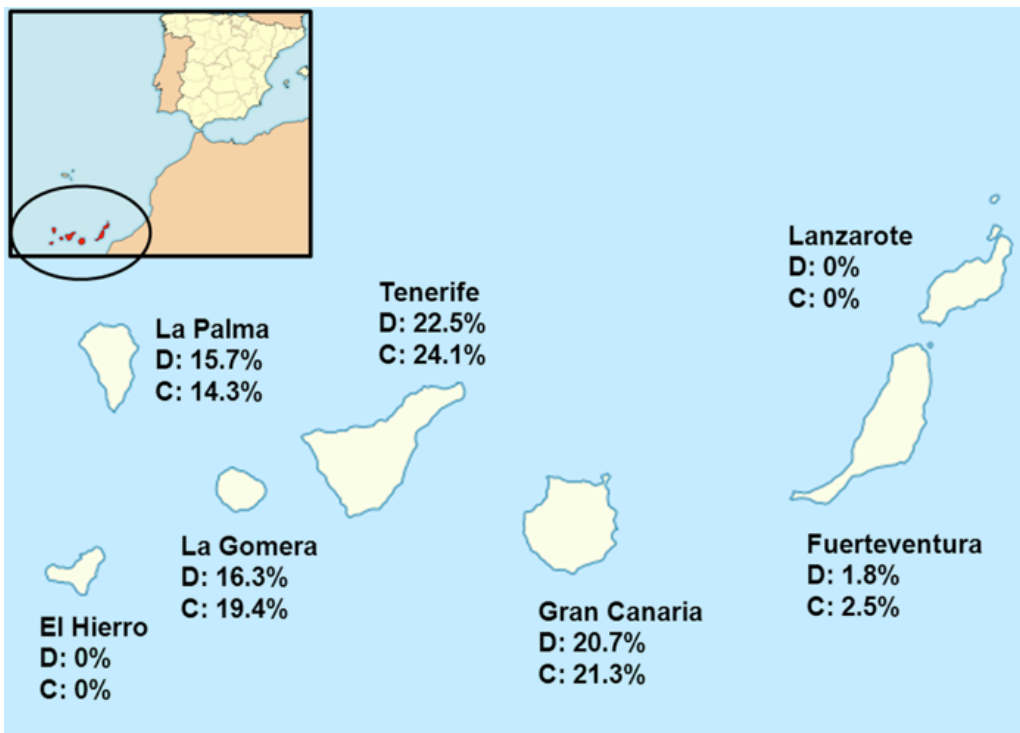
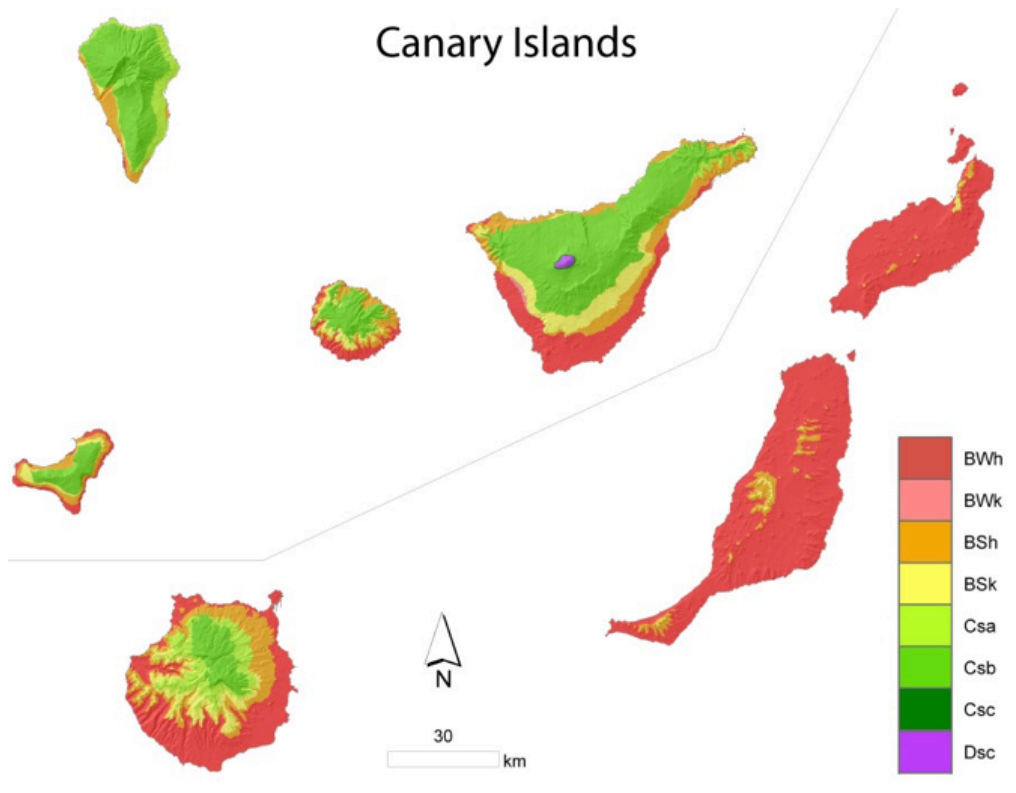
CATS	Tenerife	Gran Canaria	La Gomera	La Palma	El Hierro	Fuerteventura	Lanzarote
BW	33.3% (2/6)	19.8% (38/192)	0% (0/0)	--	0% (0/4)	2.5% (1/40)	0% (0/54)
BS	8.1% (5/62)	25.9% (15/58)	22.2% (2/9)	11.1% (1/9)	0% (0/0)	--	--
CSa	39.1% (9/23)	22.8% (16/70)	23.8% (5/21)	14.9% (7/47)	0% (0/6)	--	--
CSb	32% (24/75)	16.7% (3/18)	0% (0/6)	0% (0/0)	0% (0/7)	--	--

**Table 2.** Canine prevalence (%) and feline seroprevalence (%) by climates in the Canary Islands according to the Köppen Climate Classification. (positive animals/total animals studied) (--): No climate present in the island or presence of the climate not significant (BW: desert climate, BS: steppe climate, Csa: temperate with hot and dry summer, Csb: temperate with dry and warm summers).

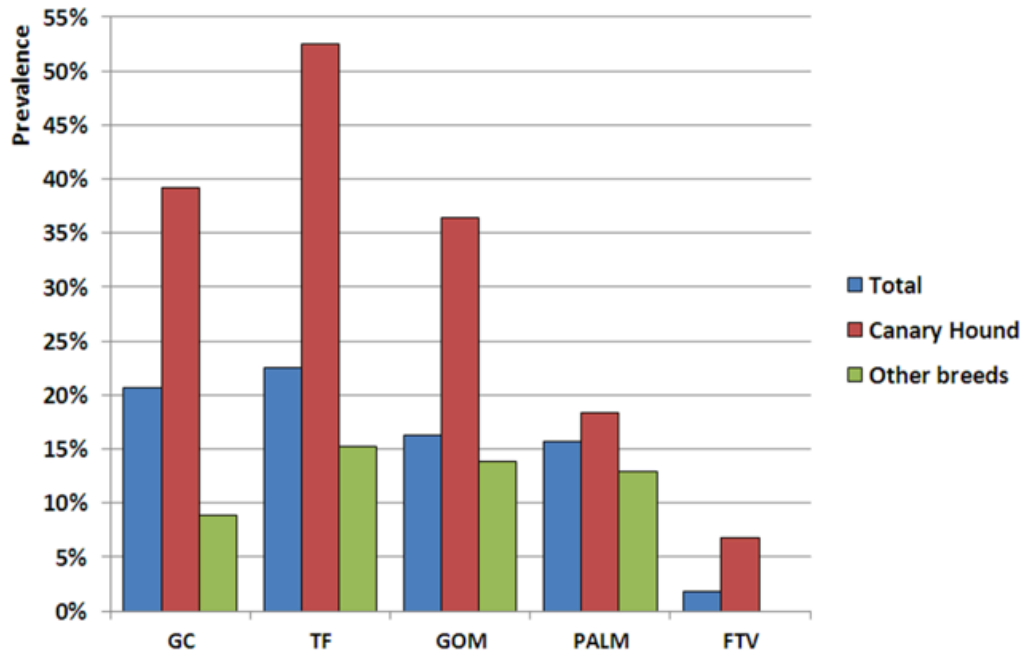
## FIGURES

**Figure 1.** (Next page up) Köppen-Geiger climate classification of the Canary Islands. Legend: BWh (hot desert), BWk (cold desert), BSh (hot steppe), BSk (cold steppe), Csa (temperate with hot and dry summer), Csb (temperate with dry and warm summers), CSc (temperate with dry and cool summers), DSc (cold without a dry season and a fresh summer). Map extracted from the Climate Atlas of the Archipelagos of the Canary Islands, Madeira and the Azores (Production Department of the State Meteorological Agency of Spain, 2012).

**Figure 2.** (Next page down) Geographical location of the Canary Islands and prevalences obtained in each islands (D: dogs, C: cats).







**Figure 3.** Canine seroprevalence of *Dirofilaria immitis* on each island with presence of heartworm infection, comparing total canine population, only the Canary Hound and other breeds of dogs except the Canary Hound. There were statistically significant differences between the prevalences of the Canary Hound and other breeds and between the Canary Hound and the total number of dogs in Gran Canaria, Tenerife and La Gomera ( $p < 0.05$ ). In Fuerteventura, there were statistically significant differences between the prevalences of the Canary Hound and the total number of dogs ( $p < 0.05$ ). Legend: (GC: Gran Canaria; TF: Tenerife; GOM: La Gomera; PALM; La Palma; FTV; Fuerteventura).



## CONCLUSIONES

**Primera.** La prevalencia de *Dirofilaria immitis* en la isla de Gran Canaria, en 1994, fue del 67,02%, en 1995 del 58,92% y en 1996 del 52,18%, con una media en los tres años del 58,89%; siendo, en ese periodo de tiempo, la más alta de España y de las más altas del mundo.

**Segunda.** La prevalencia de dirofilariosis cardiopulmonar canina en la isla de Tenerife, durante los años 2002-2003, fue del 21%. La enfermedad fue más frecuente en perros mayores de 6 años. Las diferencias por género y zonas isoclimáticas no fueron significativas.

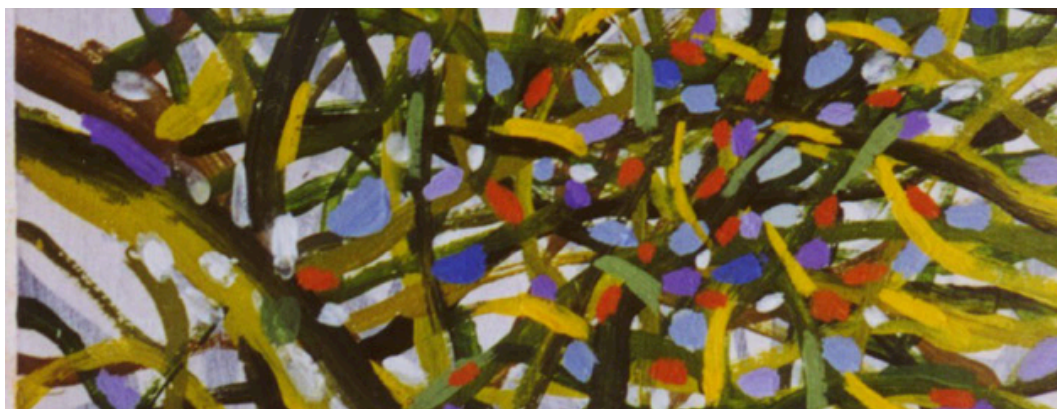
**Tercera.** En la isla de Gran Canaria, la prevalencia de *D. immitis* canina disminuyó del 30,19% en el año 2000 al 19,36% en el año 2008. En los años estudiados, la prevalencia de *D. immitis* en el podenco canario fue significativamente más elevada que en el resto de la población canina. Por lo que se puede considerar al podenco canario como un reservorio natural de la enfermedad en la isla.

**Cuarta.** En las diferentes zonas isoclimáticas de la isla de Gran Canaria, encontramos una significativa correlación entre la prevalencia de *D. immitis* canina y la seroprevalencia humana (19,36% vs 18,66%) en el año 2008. Estos datos confirmaron el riesgo zoonótico de la enfermedad en la isla.

**Quinta.** En el año 2010, la prevalencia de dirofilariosis cardiopulmonar en perros en Gran Canaria fue del 19%, mientras que la seroprevalencia en gatos fue del 33% y en humanos del 12%. Los valores de seroprevalencia mostraron una significativa correlación con los datos de prevalencia canina en las diferentes zonas isoclimáticas de la isla.

**Sexta.** En los años 2014-15 encontramos que la distribución del parásito en las islas Canarias fue heterogénea y relacionada con el clima, factores demográficos y de manejo de los animales. Como consecuencia de ello, la prevalencia de dirofilariosis canina fue del 15,7% y la seroprevalencia felina fue del 18,1%. En las islas de El Hierro y Lanzarote no se encontró ningún animal positivo, mientras que las prevalencias fueron muy bajas en Fuerteventura (1,8% perros y 2,5% gatos). En las otras 4 islas (Gran Canaria, Tenerife, La Palma y La Gomera) la dirofilariosis cardiopulmonar mostró un patrón endémico.





## RESUMEN/SUMMARY

### **Resumen**

*Dirofilaria immitis* es un nematodo de distribución mundial que afecta principalmente al perro y gato, se distribuye fundamentalmente en las zonas templadas y es una zoonosis en expansión.. Las islas Canarias se consideran una zona endémica de la enfermedad.

Con el fin de conocer la situación epidemiológica de la enfermedad en el archipiélago Canario desde 1994 se han estudiado y analizado 6860 perros, 816 gatos y 593 sueros humanos.

En la isla de Gran Canaria, la prevalencia de la dirofilariosis canina ha descendido no uniformemente de un 67,02% en 1994 hasta un 20,70% en el año 2015. En la raza podenco canario estas cifras siempre fueron más elevadas, constituyendo un reservorio natural de la enfermedad en la isla. La seroprevalencia en los gatos ha oscilado entre un 33% en 2010 hasta un 21,30% en 2015. La seroprevalencia en humanos en 2008 se situó en 18,66% y en 2010 en 12%. Tanto en gatos como en humanos encontramos una correlación con la enfermedad canina en las diferentes zonas isoclimáticas. Existe un evidente riesgo zoonótico en la isla.

En la isla de Tenerife, la prevalencia de *D. immitis* en perros fue del 21%, en los años 2002-03 y en 2015 fue del 22,5%; en gatos, este mismo año, la seroprevalencia fue del 24,10%.

En la actualidad, en las islas de El Hierro y Lanzarote no se encuentran animales positivos a la enfermedad y menos del 2% en la isla de Fuerteventura, mientras que las islas de La Palma y la Gomera muestran un patrón endémico de la parasitosis en pequeños animales.

### **Palabras clave**

*Dirofilaria immitis*, Islas Canarias, Zoonosis.

**Summary**

*Dirofilaria immitis* it is a worldwide distributed nematode which mainly affects domestic dogs and cats, fundamentally distributed in temperate areas and, furthermore, it is an emerging zoonosis. The Canary Islands are considered an endemic area of the disease.

In order to know the epidemiological situation of the disease in the Canary Islands from 1994, 6860 canine, 816 feline and 593 human sera have been studied and analysed.

On the island of Gran Canaria, the prevalence of canine dirofilariosis has decrease heterogeneously from 67.02% in 1994 to 20.70% in 2015. In the Canary Hound, these prevalences had always been higher, being a natural reservoir of the disease on the island. The feline seroprevalence has varied from 33% in 2010 to 21.30% in 2015. Human seroprevalence was 18.66% in 2008 and 12% in 2010. In both cats and humans, we found a positive correlation with the canine prevalence in the different isoclimate areas. There is an evident risk of zoonotic infection on the island.

On the island of Tenerife, the prevalence of *D.immitis* in dogs was 21% in 2002-03, and in 2015 the prevalence was 22.5%; in cats, this same year the seroprevalence was 24.10%.

Currently, in the islands of El Hierro and Lanzarote no positive animals have been found and less of 2% on Fuerteventura island, while La Palma and La Gomera have shown an endemic pattern of heartworm in small animals.

**Keywords**

*Dirofilaria immitis*, Canary Islands, Zoonoses.