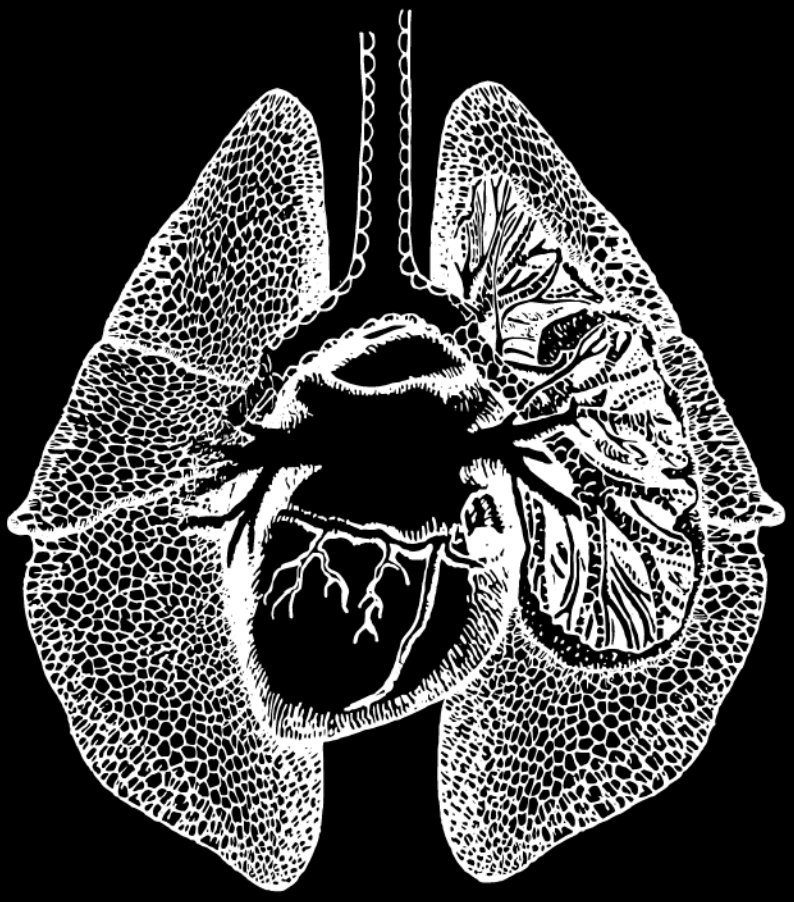

Doctorada de Investigación en Biomedicina
Tesis Doctoral con Mención Internacional
Diciembre del 2024

Radiographic Markers of Pulmonary Hypertension in Dogs and Vascular Alterations in Cats with Heartworm Disease (Dirofilaria immitis)

Marcadores Radiográficos de Hipertensión Pulmonar en Perros y Alteraciones Vasculares en Gatos con Dirofilariosis (Dirofilaria immitis)

Soraya Falcón Cordón

ULPGC
Universidad de Las Palmas de Gran Canaria
Escuela de Doctorado

ULPGC Universidad de Las Palmas de Gran Canaria | Escuela de Doctorado

PROGRAMA DE DOCTORADO DE INVESTIGACIÓN EN BIOMEDICINA

TESIS DOCTORAL CON MENCIÓN INTERNACIONAL

MARCADORES RADIOGRAFICOS DE HIPERTENSIÓN PULMONAR EN PERROS Y ALTERACIONES VASCULARES EN GATOS CON DIROFILARIOSIS (*Dirofilaria immitis*)

RADIOGRAPHIC MARKERS OF PULMONARY HYPERTENSION IN DOGS AND VASCULAR ALTERATIONS IN CATS WITH HEARTWORM DISEASE (*Dirofilaria immitis*).

DOCTORANDA:
SORAYA FALCÓN CORDÓN

DIRECTORES:
ELENA CARRETÓN GÓMEZ
JOSÉ ALBERTO MONTOYA ALONSO

LAS PALMAS DE GRAN CANARIA,
OCTUBRE DE 2024

PROGRAMA DE DOCTORADO DE INVESTIGACIÓN EN BIOMEDICINA

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LA DOCTORANDA:

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LAS PALMAS DE GRAN CANARIA,
OCTUBRE DE 2024

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INFORMA,

Que Dª Soraya Falcón Cordón, licenciada en Veterinaria, ha realizado, bajo mi dirección y asesoramiento, el presente trabajo de tesis doctoral con mención internacional titulado: MARCADORES RADIOGRAFICOS DE HIPERTENSIÓN PULMONAR EN PERROS Y ALTERACIONES VASCULARES EN GATOS CON DIROFILARIOSIS (*Dirofilaria immitis*) (*RADIOGRAPHIC MARKERS OF PULMONARY HYPERTENSION IN DOGS AND VASCULAR ALTERATIONS IN CATS WITH HEARTWORM DISEASE* (*Dirofilaria immitis*), que considero reúne las condiciones reglamentarias y de calidad científica necesarias, para su presentación y defensa, para optar al título de doctora con mención internacional por la Universidad de Las Palmas de Gran Canaria.

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ELENA CARRETÓN GÓMEZ, Doctora en veterinaria y profesora titular de Medicina y Cirugía Animal del Departamento de Patología Animal, Producción Animal, Bromatología y Tecnología de los Alimentos de la Facultad de Veterinaria de la Universidad de Las Palmas de Gran Canaria

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Passionate about animal care

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enquiries@barton-vets.co.uk

Email address:siobhan.park@cvsvets.com

Me, Siobhan Park, as Practice Director of Barton Veterinary Hospital.

I certify:

Soraya Falcon Cordon has been shadowing and coming to the practice at Barton Veterinary Hospital from the 4th of July until the 4th of September of 2023.

She has been collaborating doing research and assisting in the Cardiology Service where she has been performing:

- Echocardiograms
- Thorax x-rays
- Electrocardiography
- CT scans
- Managing Cardiac emergencies
- Taking care of cardiac in patients.

As a clinical coach in the practice, you could ask for references from Dr. Yaiza Falcon Cordon or Dr. Christopher Little.

In Canterbury at 4th of September of 2023.

Siobhan Park
Practice Director

CVS Group
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Siobhan Park

22nd April 2024

I certify that;

Soraya Falcon Cordon has been shadowing and coming to the practice at Bartons Veterinary Hospital from the 11th of December 2023 until the 5th of January 2024.

She has been collaborating and assisting in the Cardiology Service where she has been performing:

- Echocardiograms
- Thorax x-rays
- Electrocardiography
- CT scans
- Managing Cardiac emergencies
- Taking care of cardiac in patients.

References can be requested from Dr. Yaiza Falcon Cordon;

Siobhan Park
Practice Director

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4th September 2024

I certify that;

Soraya Falcon Cordon has been shadowing and been attending Barton Veterinary Hospital from 31st July until 27th August 2024.

She has been collaborating and assisting in the Cardiology service where she has been performing;

Echocardiograms

Thorax X-Rays

Electrocardiology

CT Scans

Assisting in the management of cardiac cases

Taking care of cardiac inpatients

References can be requested from Dr. Yaiza Falcon Cordon

S Park

Siobhan Park

Practice Director

Agradecimientos

Me gustaría comenzar los agradecimientos de esta tesis con un momento que marcó un antes y un después para mí y, por supuesto, las filarias son las protagonistas. Sucedió un día cualquiera, martes o jueves durante el curso. Acabábamos de llegar de pasar consultas el grupo de becarios que en aquel momento éramos Yaiza, Samantha y yo. Era la 13:00 de la tarde, la jefa estaba en el despacho tecleando en el ordenador, concentrada preparando el próximo artículo del equipo cuando nos vio entrar. No sé cómo acabamos llegando a ese punto, pero de repente, empecé a relatarles, a todos los que estaban allí, el sueño que había tenido la noche anterior. Quienes me conocen saben que soy sonámbula y suelo soñar. En mi sueño estoy en mi casa sola y de repente tocan a la puerta y, como si de una película de suspense de los años cuarenta se tratase, a través de la mirilla puedo ver un ser extraño que no logro identificar. No podía verlo con claridad porque llevaba una gabardina, sombrero y gafas de sol para no ser reconocido. Decidí abrir la puerta y, en aquel momento, pude reconocer con mucho asombro que se trataba de *Dirofilaria immitis*. Perpleja, no sabía por qué motivo se encontraba ese ser tan extraordinario en mi puerta. Antes de poder abrir la boca para preguntar, la susodicha abruptamente me dice: ¿Dónde está Elena? Yo, estupefacta y maravillada a la vez, solo pretendía retener a ese bicho cerca de mí. Tardé en responderle que Elena ha marchado de vacaciones a Bilbo y que no volverá hasta pasado el verano. *Immitis*, muy contrariada, me dice que tengo que contactarla lo antes posible, que solamente ella puede ayudarla y se trata de un asunto de gran urgencia. La invito a pasar a casa; estaba absorta en su estructura, en su andar; casi levitaba, no se quitaba la gabardina, ni el sombrero, ni las gafas de sol, algo extraño teniendo en cuenta que era pleno agosto. Le ofrezco agua o café; tonta de mí, me responde que solo toma sangre y no me deja seguir con la conversación. Quiere explicarme el motivo de su visita. Explica que la situación ha de ser resuelta lo antes posible, ya que sabe de primera mano que un familiar directo suyo pretende colonizar las Islas Canarias y que no piensa permitirlo. Ella, *Dirofilaria immitis*, es un ser endémico en estas tierras y pretende seguir siéndolo. Le pregunto quién es el invasor al que se refiere y me explica que es su prima hermana, *Dirofilaria repens*, que desde tiempos inmemoriales siempre ha querido lo que no le pertenece. Mi mente empezó a conectar sucesos y hechos, y llegué a la conclusión de que siempre la explicación más simple es la verdadera. Efectivamente, ese era el motivo por el que en nuestras Islas predomina *Dirofilaria immitis* y no *repens*. Yo no me lo podía creer, tenía que llamar a los jefes lo antes posible y avisar al equipo. Esto tenía que publicarse, dar a conocer el motivo. Estaba ante un momento histórico.

En ese momento, yo, eufórica, salgo de mis pensamientos por el ruido de la puerta. Tocan insistentemente y voy a abrir. Me encuentro otro ente filaroideo con gabardina, pero este un poco más menguado que el anterior. Se trataba de *Dirofilaria repens*. Efectivamente, amigos: pleno. Tenía en mi casa a dos filarias. Qué alegre estaba en ese momento. Pero la alegría no duró mucho, porque acto seguido ambas dos salen por la puerta de casa discutiendo sobre cómo repartirse el mundo, y a espera de que Elena pusiese orden entre ellas a la vuelta de las vacaciones. En ese momento, me di cuenta de que las filarias habían cambiado mi vida y mis sueños.

Al inicio de este sorprendente camino, no tenía ni idea de nada. Empecé porque mi hermana Yaiza comenzó con un contrato con el equipo de Cardiología de la facultad y siempre se ha sabido que, si trabajabas con Alberto, Cande y Elena, trabajabas en un sitio top. A medida que me fui sumergiendo en su mundo, me transmitieron esa pasión por las filarias. He aprendido muchísimo con ellos, pero no solo de las filarias, sino de la vida en general.

En estas palabras que voy a dedicar a continuación, me gustaría mencionar a aquellas personas que han contribuido enormemente en mi transcurso de casi 9 años de batalla, que no es poco. Empezando por mis profesores de carrera, sobre todo Cande, Zoilo Pestano, Nicanor, Begoña, Inma… y muchos otros. También me gustaría dar las gracias a otras maestras de profesión de las cuales he aprendido mucho: las mejores técnicas de laboratorio que conozco son de María, que constantemente nos ayuda con todo y trabaja como la que más dentro del equipo. Estrella, Esther y Judith. Gracias, porque siempre es un placer trabajar con ustedes y me han visto crecer dentro de esta facultad.

También quiero dar las gracias a todos esos compañeros que han pasado y con los que he podido disfrutar de esta experiencia: Samantha, Alfonso, Tania, Alba, Ainhoa, Marisa, Paula, Jorge, Sara, Bernardo, Dani, pero en especial a Noelia, que considero a día de hoy más que una amiga.

Tampoco puedo olvidarme de mi Raque, mi bichota, mi compañera de batallón durante estos últimos meses. Nunca olvidaré tus palabras, tu apoyo; más que compañera de camino, has sido mi psicóloga, mi 'cheerleader', mi polifacética e ideal amiga. Gracias por acompañarme en la peor parte; me siento afortunada de haber podido contar contigo en el trance y compartir ratos de lloros, disociación, cansancio, extenuación, pero también aprendizaje, risas, amistad, compañía y mucho cariño y comprensión de quien solo entiende lo que es pasar por esto.

No me puedo olvidar de mi queridísima Marta, compañera curtida desde hace muchos años, pero que en estos últimos nos hemos unido más si cabe. Nos hemos visto en las duras y en las maduras, y qué alegría siento de decir que he contado contigo siempre, así que esto ya no hay nada que lo separe; para siempre en mi corazón.

También me gustaría agradecer a todas las personas que me han ayudado en el camino y han sido un ejemplo de resiliencia, trabajo, constancia, gentileza, cariño, de los que te dan ejemplo de humanidad y rectitud: Rito, Marta, Antonio, Paco, Loli, Nua, Marisol, Auri.

Gracias, Rita, porque no perteneces a este mundo de la veterinaria, pero contigo he tenido el ejemplo de mujer inteligente, trabajadora, graciosa hasta decir basta; un gran apoyo para mí, sin duda. Has sido mi jefa, pero los mejores momentos que recuerdo contigo son los de la amistad con palabras gigantes. Gracias por estar en mis no tan buenos momentos y por ser parte de mi vida; tú eres una de las buenas personas que tengo a día de hoy. A ti también, Cristian, mi Cris. Gracias por estar conmigo en el camino, siempre ayudándome, dándome ánimos y acompañándome en este momento; para mí eres como un hermano.

Gracias a mis padres y mis hermanos por su cariño y apoyo, siempre sosteniéndome, cuidándome y soportando todo lo que me he propuesto. Siempre dándome lo mejor que tienen para poder conseguir mis metas. Gracias por hacerme ver la importancia de la constancia, esfuerzo, la importancia de esperar y ser fiel a tus principios. Gracias, porque sin ellos todo esto no hubiera sido posible. Por ayudarme a no desistir, tener paciencia y voluntad. Gracias por fortalecerme mentalmente y hacerme la persona que soy hoy en día. 9 años no lo aguanta cualquiera, pero nosotros no somos cualquiera.

Bueno, hemos llegado a EL PÁRRAFO. Sí, estas líneas van para ti, Yaiza. Solo puedo darte las gracias por todo, por existir, por siempre estar a mi lado. Por ser el espejo donde me reflejo, porque siempre has sido mi mejor referente y mi mejor contrincante. Porque así somos, el yin y el yang, opuestas y complementarias, pero siempre de la mano en todo lo que hacemos. Gracias por soportarme, gracias por estar presente, aún en la distancia. Gracias porque tú eres el único

ser de la faz de la tierra que me comprende perfectamente. Y sí, siempre he sabido y sabré desde el momento que vine a este mundo, primero que tú por un minuto de diferencia, que juntas podemos con todo lo que nos propongamos. Expusiste primero la tesis, pero la primera carrera sabemos quién la ganó y, obviamente, si de existir y de nacer se trata, sabes que ese vale por dos. Nunca admitiré que te empujé para salir primera; si hubo testigos, no vivieron para contarlo. Siempre instándonos a mejorar, a competir, pero con humildad sabemos que una no avanza si la otra queda atrás porque tus logros son los míos y los míos los tuyos. La motivación para terminar lo que empecé ha sido gracias a ti. Eres mi piedra angular.

No se me olvida dar las gracias a otra parte muy importante del equipo y de los que siempre que he tenido oportunidad he absorbido todo lo posible, pero sobre todo disfrutado de su compañía: Alicia, Rodrigo, Laín. Muchas gracias por formar parte de mi andar.

Por último, y muy importante, a mis jefes, a los jefes, a los jefazos. A mis pilares, mis fundamentos básicos de la veterinaria. Pero no solo de la veterinaria. Como dice la frase: "No hay consejo como el buen ejemplo", y yo de eso he tenido los mejores. Gracias, Cande, por esas prácticas de granja y de laboratorio; lo feliz que fui y no lo sabía. Gracias por tu ejemplo de bondad y elegancia; espero que donde estés, estés orgullosa. Gracias por ser como una madre; seguro aún cuidando de todos. En el transcurso del tiempo, cada vez estoy más segura de que todo el tiempo contigo que pasé fue poco.

Elena, por tu solo ejemplo, por tus consejos, por tu paciencia. Gracias, jefa, de la que todos los días aprendo algo nuevo y espero poder seguir mucho tiempo más haciéndolo. Gracias por abrir camino y cuidarnos como lo haces. Gracias por ponernos confianza, enseñarme gran parte de lo que ahora me define como profesional, pero también como persona, porque creo que si no se es una persona honesta y humilde con uno mismo, no se puede ser buen profesional. Gracias, porque sé que has hecho un esfuerzo enorme para que esto saliese y no de cualquier manera, sino impecable; no eres de este planeta, jefa. Gracias por tu ejemplo y tu presencia, porque cuando las cosas se complican, hasta las filarias acuden a ti, y eso siempre será así.

Por último, gracias, jefe, jefazo, boss, creador y precursor de todo esto. Parece una frase muy fácil de escribir, pero difícil de asimilar. Sí, precursor, gracias a ti, muchos de los que hemos pasado por esta facultad somos y seremos mejores veterinarios que nuestras cabezas nos permitan ser, por tu ejemplo. Gracias a ti aprendí algo tan básico e importante como auscultar mi primer soplo. Con el primer edema pulmonar que detecté cuando empecé a trabajar en clínica, viniste a mi pensamiento; y esos recuerdos, jefe, son difíciles de borrar. Gracias por haberme dejado formar parte de este maravilloso equipo, de tu vida y de poder comprobar, conociéndote, que no eres como dicen, eres aún mejor. Gracias porque no es lo que se enseña, sino cómo se transmite. Al fin y al cabo, más que datos, a mí me has inculcado valores, de esos que no se olvidan. Ese don y ese mérito lo tienen pocas personas en el mundo.

Por último, si os queda la duda de cómo sería esa filaria con la que Soraya soñaba, existe una prueba documental, obtenida por Alfonso Cabral Naranjo, que mientras contaba la historia en el despacho y todos atendían al desenlace, supo captar mi descripción de esa filaria que tocó un día en la puerta de mi casa y siempre estará expuesta en el despacho de los becarios de nuestra facultad.

Gracias.

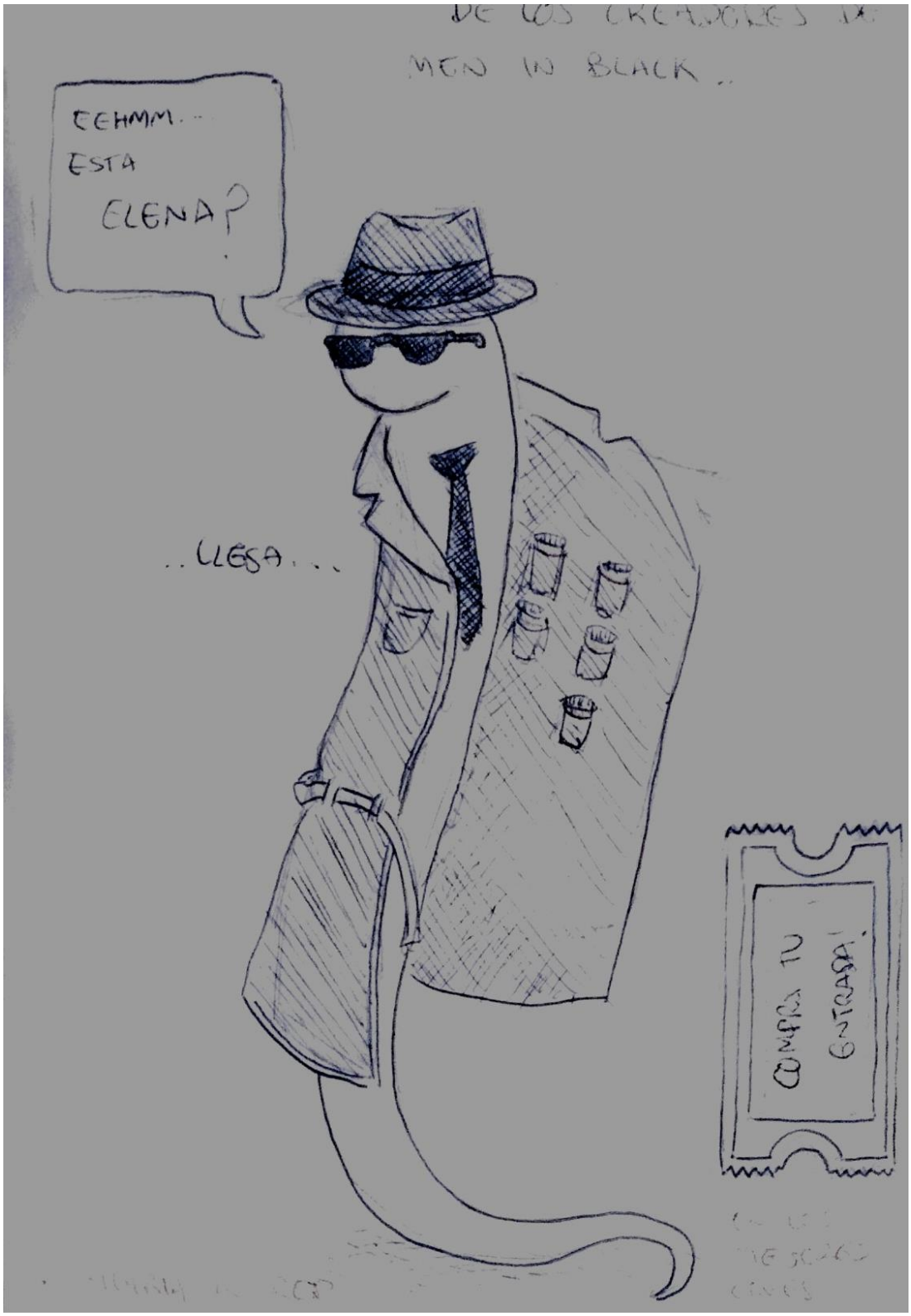

DE LOS CREADORES DE
MEN IN BLACK..
EEHMM..
ESTA
ELENA?
..LLEGA...
COMPRA TU
ENTRADA!

TABLE OF CONTENTS

FIRTS SECTION: STATE OF THE ART *DIROFILARIA IMMITIS*

INTRODUCTION

Canine heartworm disease, caused by the nematode *Dirofilaria immitis*, primarily affects domestic and wild carnivores. This parasite belongs to the genus *Dirofilaria* and resides in the pulmonary arteries and right ventricle of the heart in its adult form. The disease is transmitted by hematophagous mosquitoes, mainly from the *Culex, Aedes,* and *Anopheles* genera. The current spread of the disease is linked to several factors: the movement of infected animals from endemic to non-endemic areas, wild animals acting as reservoirs (Lee et al., 2010), and the expansion of mosquito vectors due to climate change, which favors their survival and adaptation (Anvari et al., 2019a)

In most cases, infected animals are asymptomatic or present mild clinical signs. However, once symptoms manifest, the disease can be fatal if not treated promptly (Bowman and Atkins, 2009). Adult worms can cause vascular obstruction and chronic inflammatory lesions, leading to symptoms such as chronic cough, exercise intolerance, weight loss, epistaxis, and/or dyspnea over time.

In feline species, approximately 80% of cats are asymptomatic, and the infection tends to be self-limiting. Aberrant migration of adult parasites and sudden death are more common in cats than in dogs (Lee and Atkins, 2010). Humans can act as accidental hosts for D. immitis, with an increasing number of cases of nodular lesions in the eyes, skin, and lungs in endemic areas (Genchi et al., 2009; Fontes-Sousa et al., 2019).

TAXONOMY

Dirofilaria immitis is a filiform nematode with a whitish color, belonging to the Class Nematoda, Family Filariidae, and Genus *Dirofilaria* (Urquhart et al., 2001; Marquardt et al., 2000). Within this genus, several species can parasitize dogs, including *D. immitis*, *D. repens, Dipetalonema reconditum*, and *Dipetalonema dracunculoide*, all of which produce microfilaremia during their parasitic development. Notably, *D. immitis* and *D. repens* are distinguished by their zoonotic potential and widespread distribution.

Morphologically, adult worms are cylindrical and elongated, with pronounced sexual dimorphism (Levine, 1978). Females are generally larger than males, measuring up to 300 mm in length (Manfredi et al., 2007), while males can reach up to 200 mm. Females are ovoviviparous, with the vulva located near the anterior end, where larval eggs hatch in the uterus, leading to the release of L1 (microfilariae) into circulation. Males, on the other hand, have unequal spicules and lack a gubernaculum (Mehlhorn et al., 1993).

Microfilariae measure approximately 308 μm in length, are fusiform, with a tapered cephalic end and a pointed, straight caudal end, and lack a sheath (Urquhart et al., 2001). They are released into the host's bloodstream by females and continue their development in the blood and tissues of the definitive host. Although they can be found in the blood at any time, it has been reported that their concentration in peripheral blood varies throughout the day and year (periodicity) (Léonore Lovis et al., 2017).

BIOLOGICAL CYCLE

D. immitis is a nematode with an indirect life cycle, being transmitted by vectors. The genus Dirofilaria includes numerous species, among them *D. immitis*, *D. repens*, *Acanthocheilonema dracunculoides*, and *Acanthocheilonemia reconditum*, which are notable for their incidence in Europe (Genchi et al., 2011). Due to their increasingly widespread and their zoonotic nature, the species *D. immitis* and *D. repens* have gained greater significance (Genchi et al., 2010, Simón et al., 2012).

The replication and transmission of *D. immitis* are exogenous, requiring both, a vector and a vertebrate host to complete its development. Approximately 70 species of culicid mosquitoes have been identified as potential transmitters of animal and human dirofilariasis, although in few cases has their real vector capacity been demonstrated (Cancrini and Kramer, 2001; Cancrini et al., 2006; Noack et al., 2021).

The life cycle of *D. immitis* consists of several stages. Mosquitoes during bloodsucking from an infected dog, ingest microfilariae (L1). These will develop within the

arthropod into L2 larvae and finally into L3 larvae which is the infective form, over a period of 8 to 29 days. This period fluctuates depending on environmental temperature and mosquito species (Bowman et al., 2009). Next, L3 larvae migrate to the mosquito's proboscis and are transmitted to another host through biting (Bowman et al., 2009). Once inside the definitive host, L3 larvae remain at the site of inoculation and molt into L4 larvae between 4- and 23-days post-infection. In this latter stage, they migrate to subcutaneous and intramuscular tissues before molting into juvenile (L5) stage larvae between days 50 and 58 post-infection. Finally, around day 70 post-infection, the first adult parasites (L6) will lodge in the pulmonary artery and evolve into adult forms by day 120 post-infection, beginning to replicate and releasing microfilariae into the animal's circulation around day 180 post-infection (Bowman et al., 2009).

After approximately 8 months, adult females measure between 20 and 30 cm in length. The average lifespan of the parasite is 5 to 7 years, while microfilariae can survive up to 30 months (Bowman and Atkins, 2009). Transplacental transmission of microfilariae from mother to puppies has been documented; however, L1 larvae transmitted in this way are not infective. The same occurs with microfilariae transmitted via blood transfusion (Mantovani and Jackson, 1966; Brinkmann et al., 1976; Todd and Howland, 1983; Menda, 1989).

In contrast, cats are considered susceptible hosts of *D. immitis*, but not definitive hosts. Host resistance is higher in cats, resulting in a relatively low parasitic burden of adult worms in naturally infected individuals (2 to 4 worms). The prepatent period in cats is 8 months, with microfilaremia being rare and short-lived (<20% of cats with adult worms of both sexes). Additionally, the median survival time of the parasite in cats is shorter, with a maximum of 4 years, and adult worms tend to be smaller in size compared to those in dogs.

WOLBACHIA PIPIENTIS

D. immitis harbors an intracellular symbiont bacterium known as *Wolbachia pipientis* (Sironi et al., 1995; Kozek et al., 2007). *Wolbachia* is a Gram-negative bacterium

belonging to the order Rickettsiales. These bacteria are present in all stages of the parasite's life cycle. In adult worms, *Wolbachia* is predominantly found in the cells of the hypodermis of the lateral cords, and in females, it is also present in the ovaries, oocytes, and developing embryonic stages within the uterus (Bandi et al., 1999; Kramer et al., 2003; Kozek, 2005; Sacchi et al., 2002). As a result, the bacterium is vertically transmitted from generation to generation, remaining present in all evolutionary stages of the parasite.

The presence of *Wolbachia* is essential for the survival of the parasites. Elimination of the bacteria leads to female sterility, inhibition of larval development, and the subsequent death of adult worms due to its involvement in the molting process (Langworthy et al., 2000; Gilbert et al., 2005; Dingman et al., 2010).

Upon the death of the parasite or during larval molts, *Wolbachia* bacteria are released, triggering the release of inflammatory cytokines, an increase in neutrophils, and elevated levels of specific immunoglobulins (Kramer et al., 2008). Therefore, *Wolbachia* stimulates an inflammatory response in the host organism (Morchón et al., 2004; Simón et al., 2007) and is partly responsible for the clinical manifestations of the disease (McHaffie, 2012).

EPIDEMIOLOGY

Climate and ecological factors play a direct role in the spread of vector-borne diseases across the European continent, as the development and maintenance of mosquito populations are directly influenced by climatic conditions (Genchi et al., 2011a, 2011b). Optimal temperature and ambient humidity are essential for mosquito larvae development, which, in turn, ensures the completion of the parasite's life cycle (Kalluuri et al., 2007; Genchi et al., 2009). These factors affect the seasonality of *D. immitis* transmission, with higher rates observed during the summer months (Genchi et al., 2005, 2009). Moreover, the feeding activity of mosquito species varies: *Culex pipiens* and *Anopheles* species are active only at night, whereas *An. maculipennis* and *Aedes albopictus* are active at dawn or throughout the day. Additionally, some species, like *Ae.*

caspius, show two activity peaks: at dusk and at dawn (Mattingly, 1969; Di Sacco et al., 1992; Pollono et al., 1998).

Canine heartworm disease is considered endemic in Europe (McCall et al., 2008). Over the past decade, *D. immitis* has continued to spread to Eastern and Northeastern European countries, some of which are now considered newly endemic for the disease (Morchón et al., 2022). In Southern Europe, where the disease is traditionally endemic, its prevalence continues to rise, particularly in Spain and Portugal (Genchi et al., 2020). In Portugal, an endemic area, past reports indicate that prevalence rates have remained stable or even increased in certain regions: 2.9% in the north, 2.4% in the center, and 5.1% in the south (Cardoso et al., 2012; Maia et al., 2015).

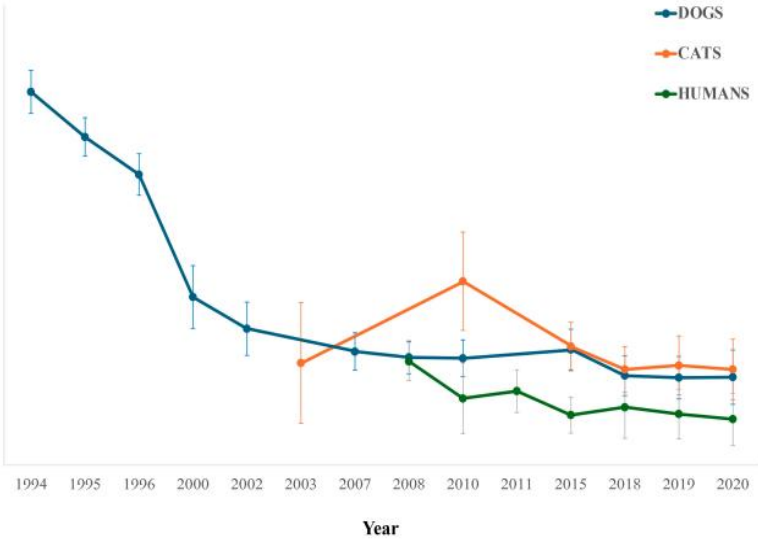
In Spain, the overall prevalence of heartworm disease ranges from 6.25% to 6.47% (Montoya-Alonso et al., 2020, 2022, 2024). In most autonomous communities, prevalence does not exceed 10%, except in the Canary Islands (11.58%) and the Balearic Islands (10.87%). Prevalence rates of 5-10% are common in many regions, with 1-5% in the northern autonomous communities.

In the Canary Islands, previous research indicated higher prevalence rates in the past, with a gradual decline in recent years, likely due to veterinary prevention campaigns (Montoya-Alonso et al., 2016), resulting in a reduction of up to 30% (Montoya-Alonso et al., 2010). However, the disease has now been reported in Lanzarote, which was previously considered heartworm-free (Montoya-Alonso et al., 2017). On the island of Gran Canaria, the prevalence of *D. immitis* in dogs was 15.81% as of 2020, while current seroprevalence in cats stands at 17.20% (Montoya-Alonso et al., 2024).

Epidemiological data in feline species indicate a higher incidence in countries where heartworm disease is traditionally endemic, such as Spain, Portugal, and Italy. Cases have also been reported in previously unexpected regions, including Germany and Austria. In Spain, overall seroprevalence for anti-*D. immitis* antibodies is 9.4%. The highest incidence occurs in the Canary Islands (19.2%), the Balearic Islands (16%), Zaragoza (24.4%), and Barcelona (11.47%). Notably, the Canary Islands have shown a progressive increase in seroprevalence, reaching 22.9% in 2022 (Montoya-Alonso et al.,

2024) (**Figure 1**). Several studies correlate feline seropositivity with canine heartworm disease prevalence (Genchi et al., 2020; Montoya-Alonso et al., 2022), estimating that feline seroprevalence is equivalent to 5-10% of the reported prevalence in dogs within the same area, potentially reaching up to 20% in specific locations (Litster and Atwell, 2008; Lee and Atkins, 2010).

Figure 1: Total Prevalence/Seroprevalence by Year in Dogs, Cats, and Humans on the Island of Gran Canaria.Source: Change in the Distribution Pattern of *Dirofilaria immitis* in Gran Canaria (Hyperendemic Island) between 1994 and 2020 (Montoya-Alonso et al., 2024)



CLINICAL SIGNS

The clinical presentation in dogs infected with *D. immitis* can vary widely, ranging from asymptomatic to severe clinical manifestations. Typically, the disease progresses slowly, and signs may not become apparent until the parasites have matured into their adult stage. Clinical symptoms may emerge as early as one year after infection but can remain undetected for several years (Kittleson, 1998; McCall et al., 2008b). The onset of clinical signs depends on factors such as parasite load, chronicity of infection, the host's activity level, and the individual host's immune response to the parasite (Dillon et al., 1995; Bowman and Atkins, 2009; Ames and Atkins, 2020).

Clinical signs develop gradually, with the most common initial symptom being exercise intolerance, followed by a chronic, non-productive cough, which can progress to moderate or severe dyspnea, fatigue, ascites, cachexia, and syncope after physical exertion (**Figure 2**) (Venco et al., 2005). Other reported signs include hemoptysis and/or epistaxis due to ruptured pulmonary vessels caused by pulmonary vascular inflammation (Carretón et al., 2012). In advanced cases, complications such as pulmonary thromboembolism, right-sided heart failure, vena cava syndrome, and death may occur (Atwell et al., 19888).

Figure 2. Dog with chronic heartworm showing severe cachexia. Source: own

During the physical examination, cardiac auscultation may reveal a systolic murmur at the right apical region in cases of tricuspid insufficiency. A diastolic pulmonary murmur may be noted in cases of pulmonary valve insufficiency. Pulmonary auscultation can detect diffuse pulmonary crackles, especially in the caudal lung lobes, although normal lung sounds do not rule out pulmonary involvement (Ames and Atkins, 2020).

In chronic cases, other clinical signs may include abdominal distension and jugular pulsation, due to increased right atrial pressure and right-sided congestive heart failure. Hepatomegaly, splenomegaly, and ascites are also common findings. Pleural effusion, pericardial effusion, or subcutaneous edema are less frequent signs, with the

latter typically associated with hypoalbuminemia (Ettinger and Feldman, 2009b; Ames and Atkins, 2020). In cases of vena cava syndrome, red blood cell lysis caused by the presence of parasites in the right atrium leads to hemoglobinuria and/or anemia.

The severity of heartworm disease in dogs can be classified based on clinical signs to determine prognosis and guide treatment. The current clinical classification system ranges from class 1 to class 4, reflecting increasing severity (Di Sacco and Vezzoni, 1992) (Table 1). Class 1 dogs are *D. immitis*-positive but asymptomatic. Class 2 dogs show moderate signs, including anemia and proteinuria, with the latter resulting from glomerulonephritis due to chronic antigenic stimulation and immune complex deposition in the glomeruli. Class 3 dogs exhibit a combination of moderate to severe clinical signs, radiographic abnormalities, and laboratory findings. Class 4 includes dogs with vena cava syndrome, typically characterized by elevated pulmonary pressures and high parasite burdens (Miller, 1998; Maxwell et al., 2014).

In feline species, the most common clinical signs are respiratory and gastrointestinal, such as dyspnea, tachypnea, coughing, vomiting, and diarrhea. Other nonspecific signs observed during physical examination include generalized weakness, weight loss, and anorexia (Pennisi et al., 2020). Additionally, the arrival and death of immature *D. immitis* forms in the pulmonary arteries induce heartworm-associated respiratory disease (HARD), which can lead to acute tachycardia, blindness, collapse, seizures, and sudden death (Dillon et al., 2014; Dillon et al., 2017b).

Table 1: Classification of symptoms during heartworm disease.

Class	Clinical signs
Mild (Class I)	Asymptomatic or cough
Moderate (Class 2	Cough, activity intolerance, abnormal lung sounds

Severe (Class 3)	Cough, activity intolerance, dyspnea, abnormal heart and lung sounds, enlarged liver (hepatomegaly), syncope (temporary loss of consciousness from reduced blood flow to the brain), ascites (fluid accumulation in the abdominal cavity), death
Caval Syndrome (Class 4)	Sudden onset of severe lethargy and weakness accompanied by hemoglobinemia and hemoglobinuria

The definitive diagnosis of the disease is based on the detection of microfilariae and circulating antigens. However, there are other complementary diagnostic techniques that provide information about the severity and prognosis of the disease that must be considered together to prepare a treatment plan (European Society of Dirofilariosis and Angiostrongylosis, 2017; American Heartworm Society, 2020).

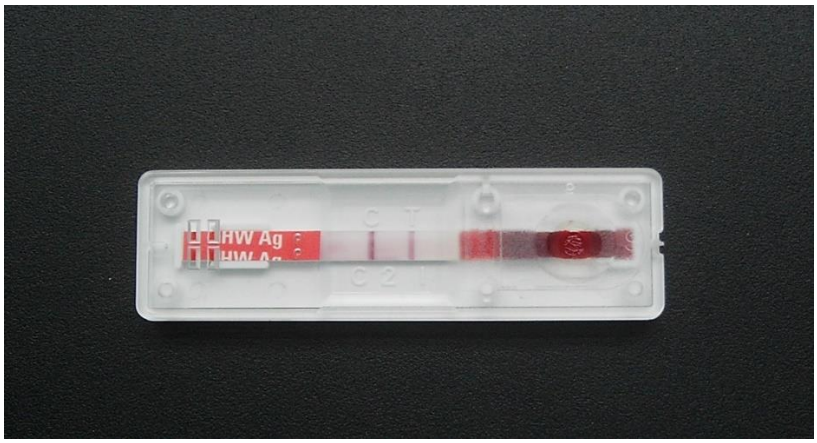
DIAGNOSIS

ANTIGEN AND ANTIBODY TESTS

The detection of circulating *D. immitis* antigens is a highly sensitive diagnostic technique (**Figure 3**). These tests are useful both in dogs presenting clinical symptoms and in asymptomatic dogs. Currently, there are several commercially available tests with varying sensitivity and specificity (Courtney and Zeng, 2001; Atkins, 2003; Lee et al., 2011). The most commonly used are ELISA (enzyme-linked immunosorbent assay) and immunochromatographic tests, which detect proteins produced in the reproductive tract of adult female *D. immitis* (Atkins, 2003; Henry et al., 2018). Circulating antigens can only be detected when female worms reach the adult stage, so antigen tests should not be performed earlier than 6 months post-infection (McCall et al., 2008). Studies have shown that the sensitivity of these tests for antigen detection depends on parasite burden, as well as the sex and age of the parasites (Lee et al.,

2011). Usually, sensitivity ranges from 90-99% and specificity ranges between 95% and 100% (Courtney, 2001; Atkins, 2003).

Figure 3: Commercial test for the detection of *D. immitis* antigens showing a positive result. Source: own



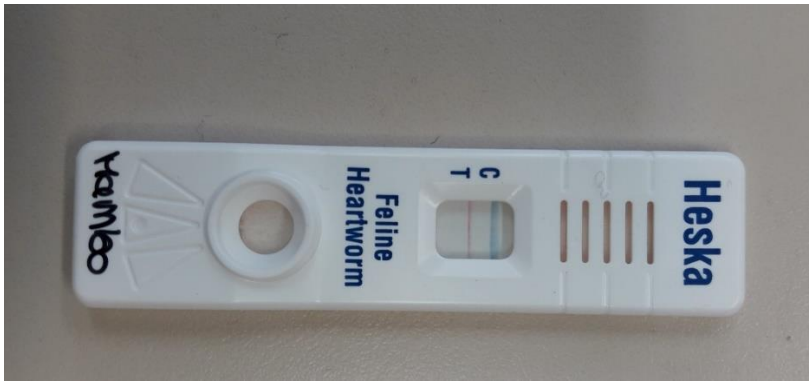
However, the inability to detect circulating antigens in dogs and cats infected with *D. immitis* has been attributed to the formation of immune complexes, which mask the antigens and lead to false negative results (Little et al., 2018). Heat treatment has been suggested to disaggregate these complexes and increase antigen test sensitivity without compromising specificity, although its use is not currently recommended for routine screening in suspected cases of *D. immitis* infection (Little et al., 2014; Beall et al., 2017; Little et al., 2018; Murillo et al., 2023). On the other hand, some authors have reported false positive results after heat treatment in dogs infected with *Angiostrongylus vasorum* and *D. repens* (Ciuca et al., 2016; Venco et al., 2017).

Diagnosing heartworm disease in cats is relatively more complex than in dogs, as cats typically have a lower parasite burden, and mono-sexual infections are more common (Genchi et al., 2008; Dillon et al., 2017a). Additionally, most infected cats are amicrofilaremic (Venco et al., 2015; European Society of Dirofilariosis and Angiostrongylosis, 2017; Pennisi et al., 2020).

For this reason, in feline species, ELISA and immunochromatographic tests for detecting circulating antigens of adult female *D. immitis* have low sensitivity. In cases of mono-sexual infections or when there is only one adult female present, ELISA and immunochromatography often yield false negative results. Therefore, a negative result

should not exclude the possibility of the infection (European Society of Dirofilariosis and Angiostrongylosis, 2017).

Figure 4: Test for the detection of anti-*D. immitis* antibodies in feline specie. Source: own



An alternative diagnostic approach in feline species is the detection of anti-*D. immitis* antibodies, which can indicate exposure to the parasite as early as two months post-infection, regardless of parasite burden (Prieto et al., 2002) (**Figure 4**). Other studies suggest that serological detection of antibodies can occur from 8 weeks to 5 months post-infection (Litster and Atwell, 2008; Lee and Atkins, 2010).

However, antibody detection tests only indicate exposure to the infection and do not distinguish between past or current infections at the time of testing. In a follow-up study of naturally infected cats with positive antibody tests, cats remained seropositive for one to three years after the initial detection (Dillon et al., 2017). Therefore, a positive antibody result does not provide information about the timing of the infection or whether the patient is currently infected with immature or adult forms of the parasite. Persistent antibodies can lead to false positives in cats that have already cleared the infection (Berdoulay et al., 2004; Venco et al., 2011).

For a definitive diagnosis of infection by adult parasites, antigen tests are recommended when clinical signs are present. However, a negative result does not rule out the disease, as it may be due to the presence of immature worms, a low parasite burden, few or no mature females, or immune complex formation that prevents detection.

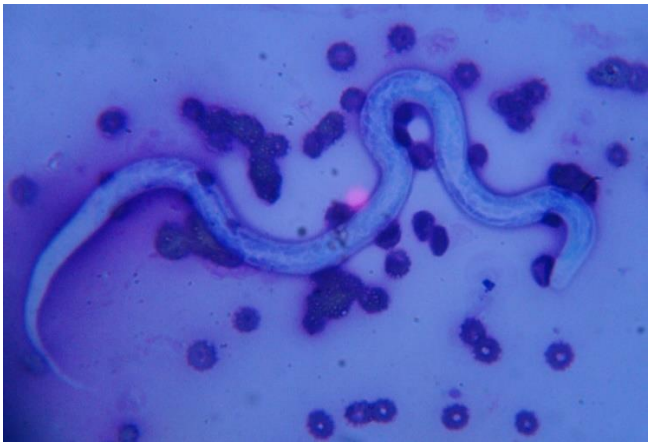
Thus, it is important to use both antigen and antibody detection tests in combination to improve diagnostic accuracy in this species. A negative result from either test alone is not conclusive and further diagnostic techniques (i.e. imaging techniques) should be carried out.

MICROFILARIA DETECTION

The detection of circulating microfilariae is a complementary method for diagnosing the disease, as 10-20% of patients can be amicrofilaremic due to factors such as mono-sexual parasitism, immature parasites, ectopic parasite location, or the destruction of microfilariae by intermittent preventive treatments or the host's immune system (Rawlings et al., 1982; Ettinger and Feldman, 2009b). However, microfilaremia detection is crucial in animals suspected of infection but with a negative antigen test result (Velasquez et al., 2014).

Several tests are available for detecting microfilariae, including direct smear, microhematocrit tube test, and the modified Knott test. The latter is the most sensitive method for detecting microfilariae, as it is a concentration technique that enables the evaluation of microfilarial morphology under the microscope, allowing differentiation between infections caused by *D. immitis* and other filarial nematodes (Knott, 1939; Rojas et al., 2015; Zajac et al., 2021) (Figure 5).

Figure 5: *D. immitis* microfilariae in a direct blood smear. Source: own



PROPHYLAXIS

Once the disease is acquired, treating it poses a significant risk to the animal, as the death of adult parasites can lead to severe complications (Miller et al., 1998). Therefore, it is recommended to initiate chemoprophylactic treatment as early as possible, ideally between 6-8 weeks of age (Atkins et al., 2011; European Society of Dirofilariosis and Angiostrongylosis, 2017; American Heartworm Society, 2020).

Macrocyclic lactones, such as avermectins (ivermectin, abamectin, selamectin) and milbemycins (milbemycin oxime and moxidectin), are the medications of choice for disease prevention. These drugs come in various formulations and application frequencies, including monthly topical or oral administration, and annual injectable forms (Prichard, 2021). The strategy of periodic administration is based on the constant exposure of dogs to mosquito bites, ensuring that no live larvae reach the pulmonary arteries, even in cases of delayed administration (Nolan and Lok, 2012).

The precise pharmacological effect of macrocyclic lactones on the various stages of the parasite is not fully understood. However, based on studies in other models, such as *Haemonchus contortus* and *Cooperia oncophora*, it is believed that these drugs act on glutamate-gated chloride channels and P-glycoprotein, which regulate the parasite's movement and reproduction (Wolstenholme et al., 2004). By binding to these receptors, the drug induces paralysis of the worm, ultimately leading to its death, as it facilitates the host's expulsion of the parasite (Prichard et al., 2001). Furthermore, some studies suggest that this class of drugs interferes with the excretory-secretory system in larval stages, reducing the secretion of immunomodulatory substances, making the parasite more vulnerable to the host's immune response (Moreno et al., 2010).

In feline species, prevention is even more critical, as adulticidal therapy is not recommended due to its high toxicity and the elevated risk of thromboembolism. Therefore, prophylactic treatment is the safest alternative to protect cats from heartworm disease (Nelson et al., 2005).

TREATMENT

Before initiating a treatment plan, several factors must be considered that influence the severity of the disease, such as the parasite burden, chronicity of the infection, the patient's activity level, and the individual response to the parasite (Bowman & Atkins, 2009). Accurately assessing each of these factors can be challenging; in many cases, dogs may exhibit symptoms beyond intermittent coughing, which could indicate chronic disease. Therefore, obtaining a comprehensive patient history and conducting a thorough physical examination are essential to rule out concurrent conditions.

The therapeutic protocol should address the different stages in which the parasite and the endosymbiotic bacterium *W. pipientis* manifest, while also aiming to prevent reinfection and treat any associated lesions.

ELIMINATION OF MICROFILARIAE AND MIGRATING LARVAE

As previously mentioned, eliminating circulating microfilariae and larvae (L3, L4) is essential not only to prevent the disease but also before initiating adulticidal treatment. This is achieved by administering macrocyclic lactones at preventive doses. Treatment should begin 2 to 3 months before administering the adulticidal drug, although this interval can be shortened to one month (Carretón et al., 2019; European Society of Dirofilariosis and Angiostrongylosis, 2017; American Heartworm Society, 2020). This approach prevents reinfection and allows parasitic forms that are not susceptible to macrocyclic lactones to continue developing into pre-adult and adult stages, which are sensitive to melarsomine dihydrochloride (Atkins & Miller, 2003; McCall et al., 2004).

TREATMENT AGAINST *WOLBACHIA PIPIENTIS*

W. pipientis, as previously described, acts as an endosymbiont that supports the parasite's development within the host. Eliminating *W. pipientis* before initiating adulticidal treatment helps prevent the inflammatory cascade (Kramer et al., 2005). Administering doxycycline at 10 mg/kg every 12 hours for 28 days reduces the occurrence and severity of pulmonary thromboembolism and associated pneumonitis

(Kramer et al., 2011; Ames & Atkins, 2020). Additionally, studies indicate that this dose can be reduced to 5 mg/kg every 12 hours with the same effectiveness (Carretón et al., 2020). Furthermore, the combined use of doxycycline and macrocyclic lactones can more rapidly reduce the number of microfilariae (Carretón et al., 2020b). It is essential to note that doxycycline treatment inhibits parasite embryogenesis, thereby reducing its survival.

ADULTICIDAL TREATMENT

The available adulticidal treatment is melarsomine dihydrochloride, administered intramuscularly in the deep lumbar region. The recommended dose is 2.5 mg/kg. The protocol consists of three injections: the first dose is followed by a second 30 days later, and the second and third doses are administered on two consecutive days. In dogs with severe infections or complications post-adulticidal treatment, administration may be postponed. This protocol kills adult worms gradually, minimizing the risk of pulmonary thromboembolism (Atkins & Miller, 2003). Side effects can occur following melarsomine administration, with the most common being mild swelling and pain at the injection site, reluctance to move due to lumbar discomfort, depression, panting, anorexia, and vomiting (Maxwell et al., 2014; Ames & Atkins, 2020).

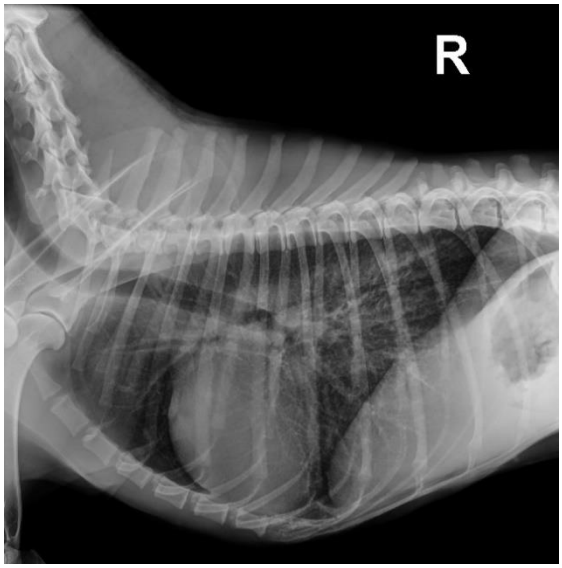
SURGICAL TREATMENT

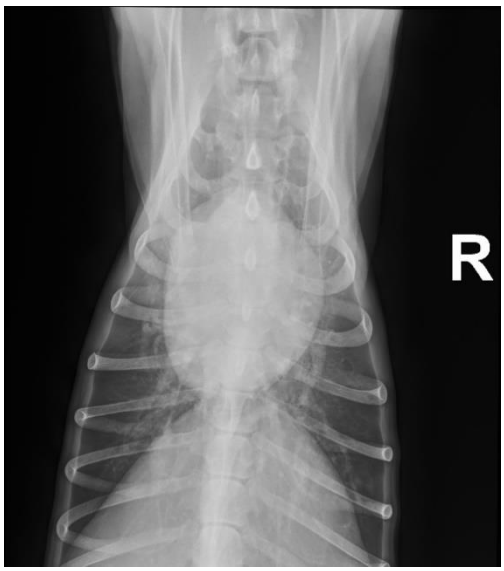
For patients with vena cava syndrome, a surgical option involves the extraction of adult worms using fluoroscopy and echocardiography. Several techniques are available, including the use of forceps, loops, or basket- or brush-type devices (Yoon et al., 2011; Saunders, 2015; Kim et al., 2023). The success rate of this treatment ranges from 50% to 67%, as many dogs with vena cava syndrome are hemodynamically unstable prior to worm extraction. However, this surgical intervention remains a viable option when euthanasia is the only other alternative (Bové et al., 2010).

TREATMENT OF PULMONARY TROMBOEMBOLISM

The treatment of canine heartworm carries a high risk of thromboembolism. After the death of adult parasites, they may embolize into the pulmonary vasculature, triggering an almost immediate inflammatory response (Sasaki et al., 1992; Carretón et al., 2013). Key clinical signs include coughing, hemoptysis, localized hemorrhages, and disseminated intravascular coagulation (Calvert & Rawlings, 1985; Ames & Atkins, 2020). Treatment primarily focuses on symptom management through the use of corticosteroids, oxygen therapy, and exercise restriction (Ames et al., 2020). The administration of corticosteroids should be carefully justified and initiated only in dogs exhibiting signs associated with thromboembolism, as inappropriate use can lead to hypercoagulability (Rose et al., 2011; Carretón et al., 2014; Ames et al., 2020).

Figures 6 and 7 Lateral-lateral and ventrodorsal radiographic projections of a patient with respiratory distress due to pulmonary thromboembolism secondary to adult worm death. Source: own





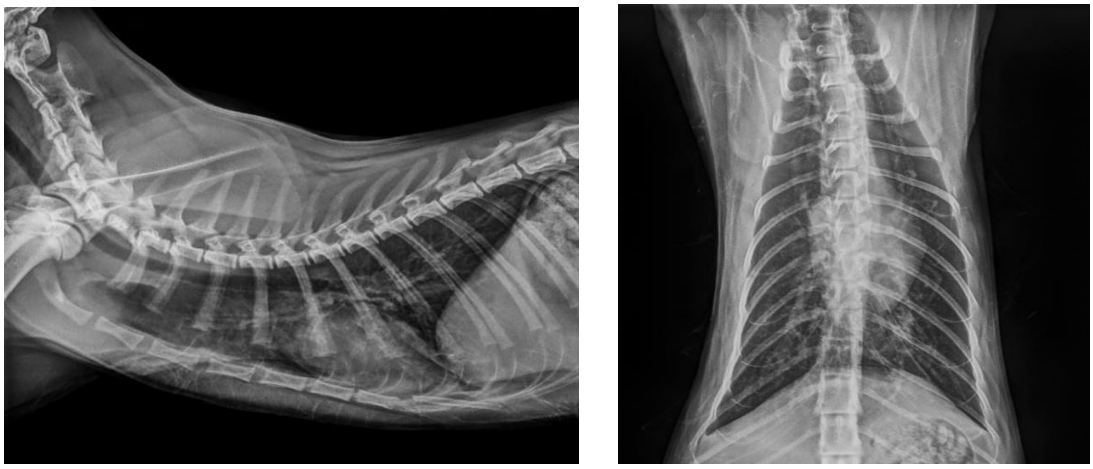
TREATMENT IN CATS

In cats, management of heartworm primarily involves supportive care, as there is currently no adulticidal treatment available that improves survival rates in affected patients. A conservative approach is typically preferred, given that the infection is usually self-limiting and often asymptomatic. Therefore, treatment is not indicated for patients without clinical signs or radiographic alterations in the pulmonary vasculature

and lung parenchyma. Periodic monitoring is necessary, with repeat antibody and antigen testing, chest radiography, and echocardiography every 4-6 months.

For cats showing clinical signs or radiological alterations indicative of lung disease, intramuscular prednisolone at a dose of 2 mg/kg, administered in decreasing doses over 4 weeks, has proven effective (**Figures 8 and 9**).

Figures 8 and 9: Lateral thoracic radiographs of a 3-year-old cat affected by immature stages of *Dirofilaria immitis*, demonstrating a diffuse, generalized bronchointerstitial pattern. Source: own



In cases with severe clinical signs, stabilization of the patient is critical and may involve the administration of fluids, intravenous corticosteroids, bronchodilators, and oxygen supplementation (**Figure 10**).

Regarding adulticidal treatment, the elimination of adult parasites is generally notrecommended, as melarsomine dihydrochloride is toxic even at low doses and has an efficacy of only 36%. Ivermectin, administered at a dose of 24 μg/kg monthly for 2 years, has been shown to reduce worm burdens by 65% compared to untreated cats; however, it carries a risk of anaphylactic reactions following the death of the parasites (Bowman et al., 2001; American Heartworm Society, 2020).

The efficacy of doxycycline in cats infected with *W. pipientis* remains uncertain, as there is insufficient evidence to suggest it has the beneficial effects observed in dogs. Consequently, doxycycline is not currently recommended as a complementary therapy for cats (European Society of Dirofilariosis and Angiostrongylosis, 2017; American Heartworm Society, 2020).

Figure 10: Cat diagnosed with heartworm, presented for consultation with acute dyspnoea. He was hospitalised with intravenous corticotherapy and monitored in an oxygen therapy chamber to observe his evolution. Source: own

CONFIRMATION OF TREATMENT SUCCESS

To confirm treatment success, an antigen test should be conducted 6 months after the last melarsomine injection. If the test remains positive, it should be repeated after another 2-3 months. It is also essential to verify that microfilariae have been eliminated. For dogs not treated with macrocyclic lactones and doxycycline (microfilariae are typically eliminated within 3-7 months), the use of a registered microfilaricide is recommended (European Society of Dirofilariosis and Angiostrongylosis, 2017; American Heartworm Society, 2020).

PROGNOSIS

In general, the prognosis is favorable for animals showing no clinical signs. Conversely, patients exhibiting severe clinical signs and advanced disease stages have a guarded prognosis. Therefore, staging the disease prior to treatment initiation is crucial to assess the risk of thromboembolism. In cases with a very high risk, modifications to standard treatment or opting not to treat may be considered (Montoya and Carretón, 2012; Romano et al., 2021). In chronic and severe parasitism, potential side effects of the disease, such as disseminated intravascular coagulation, pulmonary thromboembolism, right-sided heart failure, and pulmonary hypertension, must be considered, as some effects may be irreversible after treatment (Tudor et al., 2014; Falcón-Cordón et al., 2019).

SECOND SECTION: INVASION AND LESIONS CAUSED BY THE PARASITE

PATHOPHYSIOLOGY OF PULMONARY HYPERTENSION

PATHOPHYSIOLOGY IN DOGS

The localization of adult parasites in the pulmonary arteries induces significant lesions that affect the pulmonary parenchyma and right heart chambers (Venco and Vezzoni, 2002). These alterations result in proliferative pulmonary endarteritis, vascular remodeling, and thromboembolism, leading to sustained arterial pressure elevation and pulmonary hypertension (Atwell et al., 1988; McCall et al., 2008).

PROLIFERATIVE PULMONARY ENDARTERITIS

Inflammation in the vascular endothelium, due to the mechanical presence of adult parasites, induces anatomical changes within the pulmonary arterial wall and increases endothelial cell size (Venco and Vezzoni, 2001). These cells regulate the flow of luminal content into the perivascular tissue, facilitating the migration of smooth muscle cells to the vascular media and intima, which leads to the formation of villi on the arterial walls, causing a rough and purplish appearance (Rawlings et al., 1986; Carretón et al., 2012). These inflammatory processes result in thickening of the vascular intima, narrowing the arterial lumen (Furlanello et al., 1998; McCall et al., 2008), thereby increasing the risk of obstruction from embolization in smaller arteries (Kittleson and Kienle, 2000; Kramer et al., 2008; McCall et al., 2008b; González-Miguel et al., 2015) and elevating vascular resistance and pulmonary arterial pressure (Quinn and Williams, 2011a; Tai and Huang, 2013).

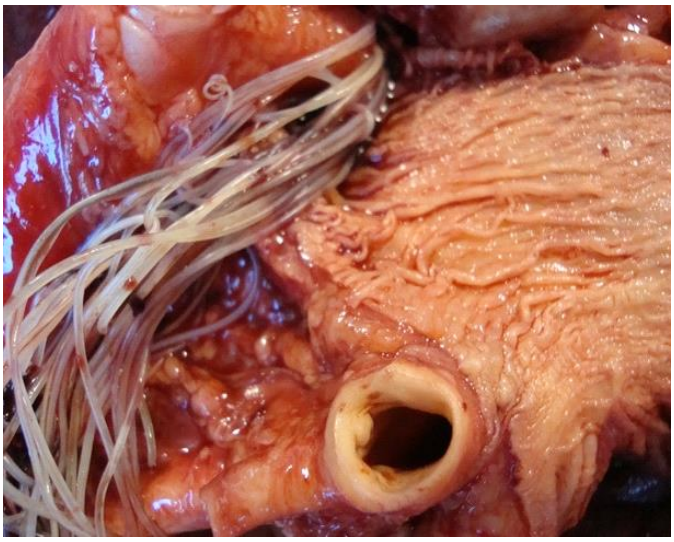
The endothelial response also includes neutrophil migration, adhesion to the endothelial surface, and platelet activation in response to endothelial damage, allowing the infiltration of albumin and other blood components into the perivascular space (Venco et al., 2014a).

Moreover, these mechanisms lead to a loss of elasticity and increased tortuosity of the affected arteries, resulting in dilation. Pulmonary arterial remodeling begins after adult parasites settle in the peripheral branches of the pulmonary artery, their primary site,

and progresses to proximal segments as the infection becomes chronic (Gómez et al., 1999) (**Figure 11**).

In addition to the endothelial response, proliferative pulmonary endarteritis is triggered by the activation of the plasminogen/plasmin system, leading to overproduction of plasmin, which degrades the extracellular matrix and promotes cell migration into the arteries, contributing to the formation of vascular microvilli. Some researchers suggest this alteration of fibrinolytic balance serves as a survival mechanism for *D. immitis*, allowing it to control clot formation in its intravascular environment, though the key factors influencing this interaction remain unknown (González-Miguel et al., 2015).

Figure 11: The image above depicts extensive parasitic infestation by adult forms of *Dirofilaria immitis* in an adult dog, along with the resulting lesions in the vascular intima caused by the parasite. Source: own



PULMONARY HYPERTENSION

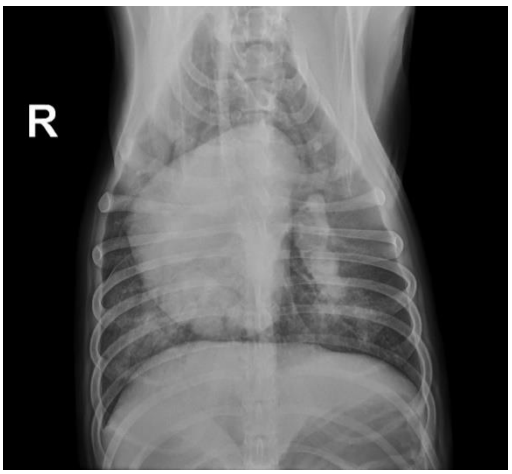
The etiology of pulmonary hypertension can be classified as primary or secondary, stemming from a series of alterations in the pulmonary vasculature that result in abnormal and persistent elevation of pulmonary arterial pressure (Serres et al., 2007; Kellihan and Stepien, 2012; Jaffey et al., 2019; Reinero et al., 2020). This increase in pressure may arise from alterations in pulmonary blood flow, pulmonary vascular resistance, postcapillary pressure, or a combination thereof (Stepien, 2009).

In the context of *D. immitis*, pulmonary hypertension is classified as precapillary, primarily caused by the adult parasite itself. This condition increases pulmonary vascular resistance and consequently elevates afterload due to vasoconstriction and vascular remodeling, leading to reduced vascular lumen, loss of elasticity in pulmonary arteries, and the formation of pulmonary thromboembolism (Reinero et al., 2020) (**Figure 12**).

The severity of pulmonary hypertension is influenced by the number of adult parasites, the duration of infection (chronicity), the host's reaction to the infection, and the level of physical activity of the affected individual (Knight et al., 1987; Dillon et al., 1995; McCall et al., 2008a; Atkins et al., 2011). However, some studies indicate that parasite burden does not always correlate with the degree of pulmonary hypertension in dogs infected with *D. immitis* (Uchide et al., 2005; Serrano-Parreño et al., 2017). In most cases, hypertension is typically moderate, although levels may increase up to threefold during exercise due to the augmented blood flow demands of the right side of the heart (Kittleson et al., 1998).

Pulmonary hypertension can present acutely in association with pulmonary thromboembolism, occurring during the sudden death of adult parasites, whether naturally or treatment-induced, resulting in arterial obstruction and vasoconstriction (McCall et al., 2008; Kramer et al., 2008; Bowman and Atkins, 2009).

Figure 12: Ventrodorsal (VD) view of canine thoracic radiographs revealing right-sided cardiomegaly consistent with *D. immitis* infection. Main pulmonary artery dilation is also evident. Source: own.



As the disease progresses, the resulting pressure overload causes hypertrophy of the right ventricle, along with fibrosis of the endocardium and subendocardium. Initially, hypertrophy is concentric and does not affect the internal diameter of the right ventricle (Hoch and Strickland, 2008; Poser and Guglielmini, 2016). Over time, however, hypertrophy becomes eccentric, leading to an increased right ventricle diameter (Nalije et al., 2013). Chronic pressure overload in the right ventricle results in increased contractility in the early stages, preserving systolic function. However, as ventricular remodeling progresses, filling capacity declines, leading to diastolic dysfunction (Gaynor et al., 2005).

Additionally, excessive adult parasites can migrate aberrantly to the right atrium, right ventricle, and often the vena cava, resulting in vena cava syndrome—a potentially life-threatening state of heart failure (Bowman and Atkins, 2009; Simon et al., 2012; Ames and Atkins, 2020). The retrograde migration of adult parasites from the pulmonary arteries to the right ventricle and right atrium is multifactorial (Strickland, 1998; Bove et al., 2010). Contributing factors may include elevated pulmonary artery pressures, decreased cardiac output, high parasite burden, alterations in parasite maturation, and/or the administration of adulticidal or preventive treatments. Clinically, this condition is characterized by acute and severe signs, although some patients may present with mild symptoms.

Regarding the role of the right atrium, some authors propose that initially, the right atrium increases contractility to facilitate right ventricle filling (atrial impulse). However, as the disease progresses, the right atrium dilates to maintain cardiac output and prevent congestive heart failure due to chronic right ventricle pressure overload (Hoit et al., 1993; Nishikawa et al., 1994). Therefore, the compensatory capacity of the right atrium is critical; in cases where this compensation fails, signs of congestive right heart failure may manifest (Laks et al., 1969; Dillon et al., 1995; Gaynor et al., 2005; McCall, 2008).

LUNG ALTERATIONS

The pulmonary vasculature is a low-pressure, high-capacity blood storage circuit. Pulmonary arteries possess significant elastic capacity, characterized by highly distensible walls composed of intima, media, and adventitia layers. The intima, in direct contact with the vascular lumen, consists of a single layer of endothelial cells, collagen, and fibroblasts. The media is thicker and composed of elastin, collagen, and smooth muscle cells, while the adventitia primarily consists of collagen (Kellihan and Stepien, 2012).

During *D. immitis* infection, several alterations occur, including concentric thickening, increased muscular layer of the intima, hypertrophy, and fibrosis of the media due to plasma and inflammatory cell infiltration. This leads to perivascular edema, inflammation, and, in advanced stages, irreversible fibrosis (Rawlings et al., 1986). Severe cases may exhibit irregular lesions and overgrowths resembling villi in the vascular intima, encroaching upon the arterial lumen. Vascular intimal wall necrosis may also be observed (Quinn and Williams, 2011a). These alterations impair gas exchange, contributing to increased vascular resistance.

Moreover, two arterial systems comprise the pulmonary circulation: the pulmonary artery, which facilitates gas exchange in the alveoli, and the bronchial artery, essential for supplying nutritional blood to the lungs. Due to peripheral pulmonary blood flow obstruction, a compensatory mechanism activates in which the bronchial artery dilates and, in some cases, proliferates via bronchopulmonary shunts to maintain left ventricular function (Kobayashi et al., 1988).

The sudden death of parasites may induce bronchial artery dilation to facilitate venous return, altering pulmonary venous circulation. This obstruction predominantly affects the right caudal lung lobe (Jerry, 1961; Wakao, 1992). In some instances, peripheral blood flow obstruction in the pulmonary system is compensated for by precapillary anastomoses formed between the bronchial artery and the pulmonary artery, promoting pulmonary hypertension in dogs with heartworm (Shibata et al., 2000).

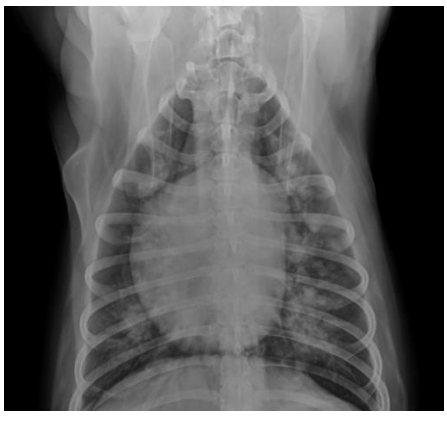
Other pulmonary lesions described in cases of heartworm include pulmonary eosinophilic infiltration syndrome (Confer et al., 1983; Atwell and Tarish, 1995; Reinero et al., 2019). This syndrome occurs in cases of hypersensitivity to antigens produced by microfilariae, where they are neutralized and rapidly destroyed in the pulmonary circulation (Calvert and Losonky, 1985). The detection of microfilariae facilitates leukocyte and eosinophil influx, resulting in granulomatous inflammation (Confer et al., 1983; Bowman and Atkins, 2009).

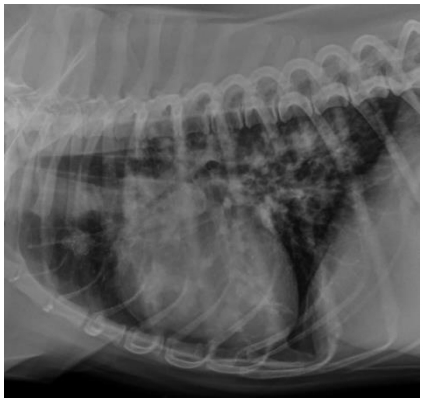
Other lesions affecting pulmonary parenchyma during infection include pulmonary thromboembolism. Smaller pulmonary vessels become obstructed following the death of adult worms and coagulation activation, restricting pulmonary circulation and leading to consolidation of the affected lobe (Bowman and Atkins, 2009).

PULMONARY THROMBOEMBOLISM

Pulmonary thromboembolism occurs due to the obstruction of one or more pulmonary vessels following the death of adult parasites. Canine heartworm is among the causal agents of pulmonary thromboembolism, resulting from alterations in one of Virchow's triad factors: hypercoagulability, blood flow stasis, or endothelial injury. In the case of *D. immitis*, endothelial integrity of the arterial wall is compromised, leading to increased blood hypercoagulability (Keith et al., 1983; LaRue et al., 1990). These conditions promote the formation of villi in the vascular intima and perivascular edema (Schaub and Rawlings, 1980) (**Figures 13 and 14**).

Figures 13 and 14: Latero-lateral and dorso-ventral thoracic radiographs revealing a pattern consistent with pulmonary thromboembolism in a dog infected with *Dirofilaria immitis*. The images demonstrate an alveolar infiltration pattern characterized by areas of opacity indicative of secondary pulmonary congestion, as well as multiple pulmonary nodules or lesions suggesting ischemia in the affected lung regions.





Moreover, fragments of the parasites are exposed to the host's immune system, becoming partially integrated into the arterial wall, where significant connective tissue is formed as scar tissue (Atkins et al., 2005). Additionally, parasites occlude smaller pulmonary vessels, with the caudal lobes being the most affected, leading to reduced or interrupted blood supply, resulting in pulmonary lobe consolidation and/or pulmonary infarction. This creates an imbalance in the ventilation-perfusion relationship and, consequently, hypoxemia (McCall et al., 2008b).

When the vascular endothelium is exposed to high doses of *D. immitis* antigens, there is increased expression of NO, eNOS, and iNOS, and an increase in inflammation-related eicosanoids, such as COX-2, 5-LO, PGE2, and LTB4 (Morchón et al., 2008). There is also increased expression of adhesion molecules ICAM-1 and PECAM-1. The parasite antigen further decreases endothelial cell permeability. Additionally, it stimulates the production of other factors such as endothelin-1 or platelet-released factors like serotonin, adenosine diphosphate (ADP), or thromboxane A2, which contribute to pulmonary arterial vascular vasoconstriction (Kramer et al., 2006; Ettinger and Feldman, 2009a).

PATHOPHYSIOLOGY IN CATS

Heartworm manifests differently in feline species, with cats exhibiting significantly lower immunological tolerance to *D. immitis* infection. There are two main presentations of the disease: one caused by immature parasites and the other by chronic disease due to adult forms. Generally, in cats, the parasitic load is low, leading to severe pathological changes that can result in high mortality and/or sudden death, especially in cases of adult parasite infection (Venco et al., 2008; Bowman and Atkins, 2009).

Adult worms cause lesions in the pulmonary arteries characterized by villous endarteritis, intimal fibrosis, medial hypertrophy, and thrombosis (Browne et al., 2005). In instances where the parasite reaches adulthood, cats may remain asymptomatic. Mature adult parasites are believed to secrete mediators that regulate the activity of

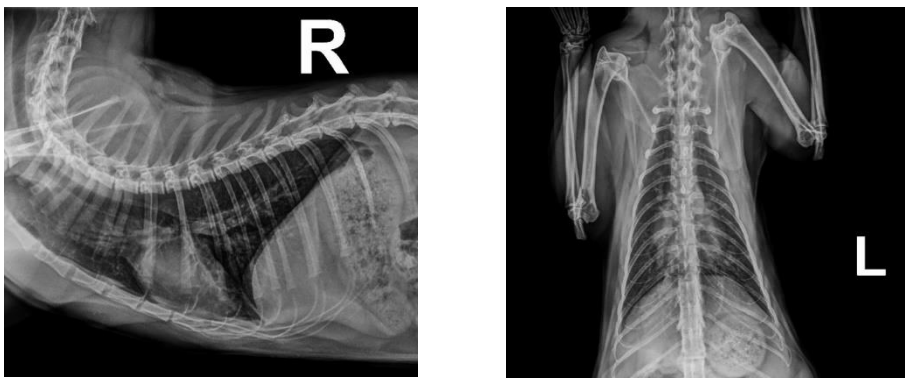
polymorphonuclear cells, resulting in an anti-inflammatory effect that minimizes clinical signs (McCall et al., 2008).

The inflammatory response becomes evident when immature worms reach the pulmonary vessels, approximately 90 days post-infection. Most immature forms are destroyed by the host within 3-4 months, preventing their development into adult forms (Dillon et al., 2007; 2014). Unlike dogs, cats possess pulmonary intravascular macrophages, which may be modulated by parasitic products, contributing to distinct pathophysiological effects in cats compared to dogs (Dillon et al., 2008).

In cases of early death of larval forms, severe acute lesions may occur, including pulmonary thromboembolism and eosinophilic inflammatory pneumonitis, leading to what is known as HARD (Heartworm Associated Respiratory Disease) (Atkins and Litster, 2006; Garcia-Guasch et al., 2013). In these cases, pulmonary disease primarily affects the bronchial, interstitial, and pulmonary artery levels (Dillon et al., 2007). Following initial infection, most cats with HARD will develop antibodies against *D. immitis* (Dillon et al., 2007).

During infection, whether in the immature stage (<180 days post-infection) or in the adult stage (>180 days post-infection), chronic bronchial damage may occur, alongside involvement of small airways and alveolar infiltration. The interlobar pulmonary branches are primarily affected, exhibiting thickened walls (Blagburn et al., 2007). Bronchial hyperreactivity and inflammation are common sequelae, characterized by excessive and reversible smooth muscle contraction (bronchoconstriction) in response to stimuli (Liu et al., 2006) (**Figures 15 and 16**).

Figures 15 and 16: Lateral and ventrodorsal radiographic view of a cat infected with *D.immitis*, showing a diffuse bronchointerstitial pulmonary pattern and pulmonary hyperinflation. Source: own



THIRD SECTION: RADIOLOGICAL FINDINGS: "EXPOSING" THE PARASITE

In human medicine, the effectiveness of thoracic radiology in diagnosing and staging cardiopulmonary diseases has been extensively evaluated in comparison to other imaging techniques (Fisher et al., 2010; Pagnamenta et al., 2015). Establishing the relationship between clinical signs and radiological findings is crucial for accurate diagnosis (Woznitza et al., 2018).

In veterinary medicine, thoracic radiography is a widely accessible and frequently employed tool in the daily clinical practice for small animals (Thrall, 2018). This cost-effective diagnostic technique is valuable for the initial investigation of a broad spectrum of pulmonary and cardiac diseases in dogs (Rudorf et al., 2008). However, interpreting these radiographs can be challenging due to variations in visible structures influenced by the animal's size, age, breed, and sex (Mai et al., 2008; Thrall, 2018). Thoracic radiography is essential for guiding diagnoses based on a clinical history indicative of cardiorespiratory disease (Pinto and Brunese, 2010; Arruda et al., 2023), although some authors note that the operator’s familiarity with clinical signs may negatively affect interpretation (Small, 2021).

Many diseases in dogs and cats lead to cardiac and pulmonary alterations, where thoracic radiography provides valuable insights into disease progression (Atkins et al., 2009; Borgarelli et al., 2015; Malcolm et al., 2018). Furthermore, when combined with other diagnostic techniques, thoracic X-rays show a high correlation in diagnosing specific cardiorespiratory pathologies, including bronchial collapse (Johnson et al., 2015), degenerative mitral valve disease (Chalermpromma & Surachetpong, 2023), and dilated cardiomyopathy. In cases of mitral valve disease and dilated cardiomyopathy, the combination of thoracic X-rays with electrocardiography is the preferred diagnostic approach (Wesselowski et al., 2022).

Specifically, in the study of pulmonary hypertension, thoracic radiography has proven to be a relevant diagnostic technique, especially in those cardiorespiratory pathologies that produce pulmonary hypertension secondary to respiratory disease and/or

hypoxia, pulmonary thromboembolism, and parasitic diseases that trigger pulmonary hypertension, such as *A. vasorum* and *D. immitis* (Reinero et al., 2020).

There are studies that have allowed radiological differentiation between animals that present pulmonary hypertension and those that do not, detecting radiological abnormalities such as dilation of the right heart chambers and the main pulmonary artery and its branches (Robert, 2007; Adams et al., 2017; Chanroon et al., 2018). Conversely, other authors state that pulmonary hypertension cannot be diagnosed solely based on radiographic findings, as they are only suggestive parameters (Kellihan & Stepien, 2010).

Furthermore, other authors indicate that determining pulmonary hypertension can be reliable in dogs and cats with at least moderate pulmonary hypertension, as they obtained a significant association directly proportional between radiological findings and the severity of pulmonary hypertension obtained through echocardiography (Adams et al., 2017). Also, another study determined a correlation between the diameter of the caudal pulmonary artery at its bifurcation and body surface area and systolic pulmonary arterial pressure (Lee et al., 2016).

Conversely, some authors pointed out that it may be useful in diagnosing the underlying cause but not for diagnosing pulmonary hypertension, as it is an unspecific and subjective diagnostic technique, even with patients with severe pulmonary hypertension, with minimal radiological changes (Ware, 2007; Kellihan & Stepien, 2010).

In dogs affected by *D. immitis*, thoracic radiology has been useful for assessing disease extent and determining the suitability for adulticidal treatment (Atkins, 2010; Tudor et al., 2014; Vetter et al., 2023). Notably, only 25-30% of affected dogs did not exhibit radiological alterations during the disease in several studies (Polizopoulou et al., 2000; Losonsky, 1983). Other studies corroborated that thoracic radiography can detect nonspecific alterations in up to 98% of patients, with cardiomegaly being the most frequently observed abnormality in 84% of animals. Moreover, 31% of dogs displayed pulmonary artery enlargement, yet no significant differences were found between

radiological findings and the severity of PH determined echocardiographically (Johnson & Orton, 1999).

RADIOLOGICAL SIGNS OF PULMONARY HYPERTENSION

CARDIAC ALTERATIONS.

Radiological evidence suggestive of pulmonary hypertension includes enlargement of the right-sided cardiac chambers and dilation of the pulmonary artery. Right-sided cardiomegaly is characterized by the rounding of the cranial and right lateral margins of the cardiac silhouette, accompanied by caudal displacement of the cardiac apex to the left, resulting in an appearance reminiscent of an inverted D-shape on a ventrodorsal or dorsoventral projection (Adams et al., 2017; Bahr, 2017) (**Figure 17**). However, this finding is unusual in feline species, where generalized cardiomegaly is more common (Johnson and Hansson, 2013). This discrepancy may be attributed to the rarity of pulmonary hypertension and right ventricular hypertrophy in cats (Small et al., 2008; Venco et al., 2015).

Several measures can be employed to determine the presence of cardiomegaly, including the Manubrium Heart Score (MHS), Heart to Single Vertebral Ratio (HSVR), and Thoracic Inlet Heart Score (TIHS). These methods have proven useful in studying cardiac silhouette (Mostafa & Berry, 2017; Costanza et al., 2023; Marbella et al., 2023). The most widely used method for assessing cardiomegaly, however, is the vertebral heart score (VHS) or Buchanan index. This method involves taking a lateral thoracic radiograph to measure the longitudinal cardiac axis (from the base of the carina to the cardiac apex) and the short cardiac axis (the maximum axis perpendicular to the longitudinal axis in the middle third). These values are then referenced to the vertebral column, starting from the anterior facet of the fourth thoracic vertebra (T4). The number of thoracic vertebral bodies corresponding to both axes is summed (Buchanan, 2000).

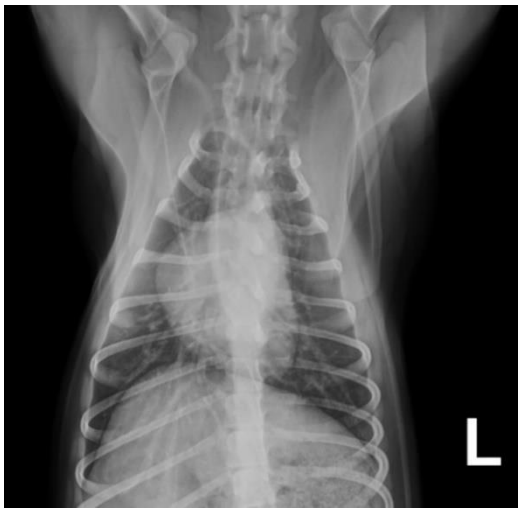
In some cases, left-sided cardiomegaly may not be apparent, leading to widening of the cardiac chambers relative to their height. This alteration can be evaluated using a

cardiac proportion of 3/5 to 2/5 by drawing a line parallel from the carina to the apex of the heart. Under normal conditions, approximately 3/5 of the cardiac silhouette area should be cranial to this line, while the remaining 2/5 should be caudal. If the cranial cardiac silhouette exceeds 3/5, right-sided cardiomegaly is indicated (Suter, 1984).

Some authors have noted that the study of cardiac remodeling may occasionally yield subjective information regarding the presence and severity of enlargement of cardiac chambers and blood vessels, particularly in cases of moderate to severe left-sided cardiomegaly (**Figures 18 and 19**). This is due to the distortion of cardiac chambers, which can create a false impression of right-sided cardiomegaly (Carlsson et al., 2009; Huget et al., 2021). Other researchers suggest that these associated alterations, such as the inverted D-shape, main pulmonary artery dilation, and signs of congestive heart failure, may manifest differently in large and small dogs. Consequently, thoracic radiography may underestimate the severity of the disease in smaller dogs infected with *D. immitis*, necessitating an echocardiographic study (So-Young et al., 2019).

Moreover, studies indicate that the Vertebral Heart Score (VHS) can vary based on the position used in the lateral projection, with higher values typically observed in the right lateral position in healthy dogs (Greco et al., 2008).

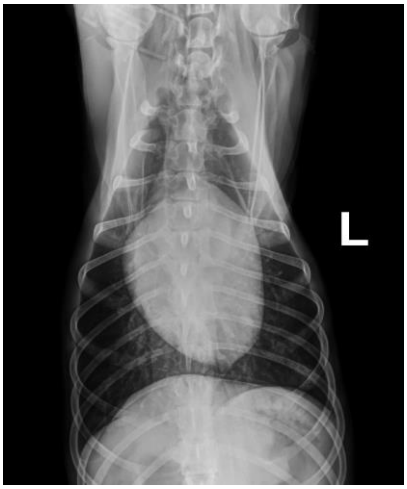
Figure 17: Dorsoventral thoracic radiography of Rumi, a 2-year-old canine patient infected with *D. immitis*, exhibiting a high parasitic load and dilation of the right cardiac chambers with the classical inverted D-shape. Source: own

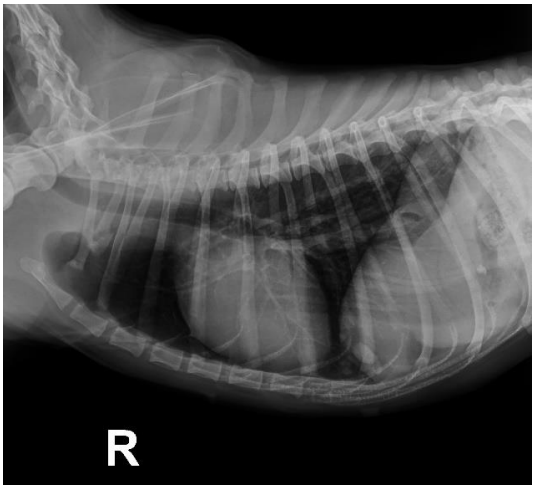


In cats, manifestations of congestive heart failure and signs of pulmonary hypertension, such as left ventricular or left atrial hypertrophy, are infrequent. Consequently, these radiological alterations are typically not evident. However, there is evidence of collagen depletion within the right ventricular myocardium, which is directly associated with the severity of pulmonary parenchymal and vascular alterations in cats affected by the immature forms of the parasite (Winter et al., 2017) (**Figure 20**).

On the other hand, the radiological detection of pulmonary artery dilation appears as a soft tissue density prominence that merges with the cardiac silhouette. For accurate assessment, the diameter of the right cranial pulmonary artery is compared to the width of the fourth right rib in a lateral projection; an index greater than 1.2 suggests pulmonary artery dilation (Thrall & Losonsky, 1976). Another quantitative technique for reliably measuring the pulmonary artery diameter involves comparing it to the width of the ninth rib in a ventrodorsal or dorsoventral projection, with a ratio greater than 1:1 being indicative of pulmonary artery dilation. In cases of chronic and severe pulmonary hypertension, pulmonary vascular tortuosity may also be detected (Bahr, 2017; Reinero et al., 2020).

Figures 18 and 19: Thoracic radiography of a dog with heartworm, in dorsoventral and lateral projections, revealing a moderate enlargement of the cardiac silhouette.

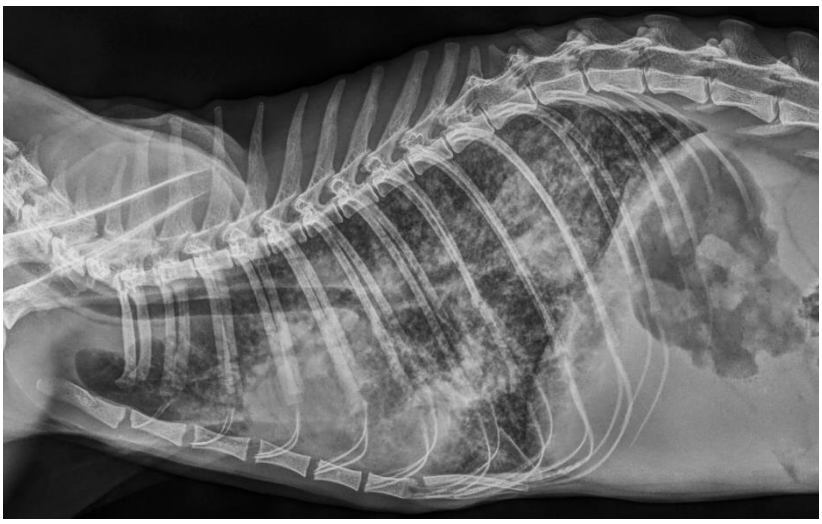




PULMONARY PARENCHYMAL ABNORMALITIES

The most common pulmonary alterations associated with *D. immitis* infection in dogs result from increased pressure, leading to enlargement and increased tortuosity of the intralobar and interlobar peripheral branches of the pulmonary arteries. Lung parenchyma lesions primarily affect the caudal lobes initially, spreading to other areas as the disease progresses (Calvert and Rawlings, 1988; Rawlings, 1986; Bowman and Atkins, 2009).

Figure 20: Right lateral thoracic radiograph of a feline patient with acute respiratory distress showing lesions consistent with heartworm-associated respiratory disease (HARD).



In dogs with chronic infection, irreversible mineral deposits occur within the pulmonary vasculature. Another sign associated with chronicity is the formation of small opaque soft tissue nodules, typically with well-defined borders, or mineral nodules indicative of granulomatosis (Johnson and Hansson, 2013). In cases of pulmonary thromboembolism, areas of increased soft tissue opacity and/or hypovascularized lucent regions are identified, predominantly with a peripheral distribution (Bahr, 2017).

In cats, pulmonary alterations are more pronounced, as *D. immitis* infection in this species induces a more significant inflammatory response in the lung parenchyma. Interstitial inflammation, along with hypertrophy and hyperplasia of type II pneumocytes and proliferation of smooth muscle cells, results in fibrosis and

emphysema of the lung parenchyma. Pneumonitis is characterized by the presence of eosinophils, macrophages, plasma cells, mast cells, and fibroblasts, indicating eosinophilic pneumonitis (Maia et al., 2011; Dillon et al., 2014).

RADIOLOGICAL AND ECHOCARDIOGRAPHIC ASSOCIATION IN HEARTWORM DISEASE.

Thoracic radiography has proven to be highly useful in diagnosing heartworm disease in dogs, with radiographic abnormalities detected in up to 74% of cases (Tudor et al., 2014). In the assessment of pulmonary hypertension, thoracic radiography is often used in conjunction with other diagnostic techniques, with echocardiography being the most commonly employed method. For pulmonary hypertension caused by *D. immitis*, the Right Pulmonary Artery Distensibility Index (RPADi) serves as a validated parameter to estimate both the presence and severity of pulmonary hypertension in dogs.

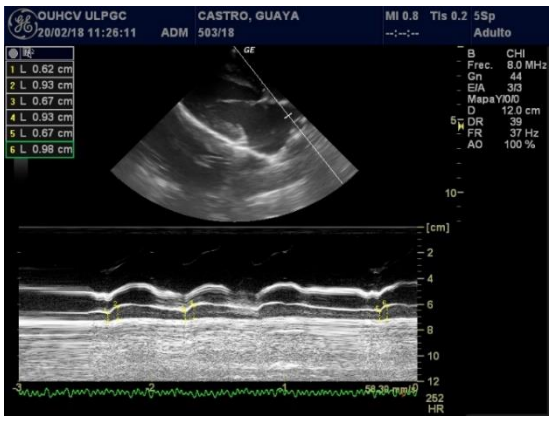
Several different methods for obtaining the RPADi have been published. One such method, proposed by Venco et al (2014), involves measuring the diameter of the right pulmonary artery from leading edge to leading edge (le-le) using a right parasternal long-axis view in M-mode (Thomas et al., 1993; Venco et al., 2014). The modified Venco method measures the diameter from trailing edge to leading edge (te-le) (**Figure 21**).

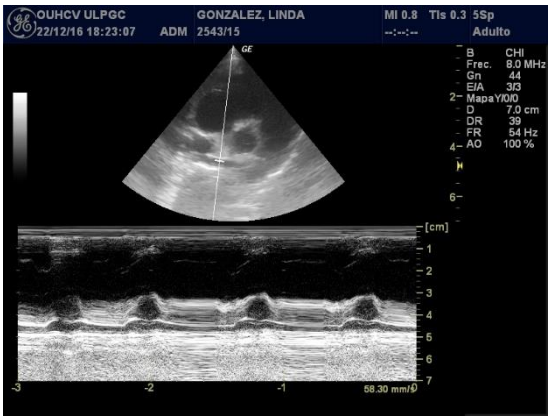
The classic Visser method also measures the right pulmonary artery from edge to edge (le-le), but utilizes a right parasternal short-axis view in B-mode (Visser et al., 2018) (**Figure 22**). Additionally, the modified Visser method, from the same view, measures the pulmonary artery from trailing edge to leading edge (te-le). Another alternative proposed by Visser et al. involves obtaining measurements from M-mode images using the edge-to-edge method.

Other hybrid methods have been reported, such as the ratio of the pulmonary vein to the right pulmonary artery, which have shown utility in evaluating pulmonary hypertension (Roels et al., 2019; Visser et al., 2020). The primary distinction between B-mode and M-mode imaging lies in the potential superiority of the M-mode method for

evaluating the RPADi, owing to its more precise delineation of the measurement points during systole and diastole, as well as its better temporal resolution compared to B-mode (Basile et al., 2023).

Figures 21 and 22: Echocardiographic images illustrating various methodologies for determining pulmonary hypertension. The first image showcases the technique proposed by Venco et al. (2014) utilizing M-mode, followed by the approach established by Visser et al. (2016) employing B-mode.





RPADi values below 29%, according to the methodology applied by Venco et al. (2014) are considered to indicate mild pulmonary hypertension. On the other hand, Visser et al. (2018) established that pulmonary hypertension was considered when this index was below 34.6%.

These measurement techniques are used alongside thoracic radiography for staging and monitoring pulmonary hypertension. Echocardiography is employed when radiological evidence indicates tortuous and/or dilated pulmonary arteries, accompanied by compatible pulmonary alterations such as localized or diffuse alveolar infiltration, and prominence in the region of the pulmonary trunk or right-sided cardiac enlargement (Reinero et al., 2020).

However, a comparative study examining the relationship between the severity of radiographic abnormalities and the severity of pulmonary hypertension as determined echocardiographically during heartworm disease has yet to be conducted.

FOURTH SECTION: AIM AND RESULTS

OBJECTIVES

Evaluate the Correlation Between Radiographic Changes and the Presence of Pulmonary Hypertension in Dogs Infected with *D. immitis*: This objective aims to analyze the association between radiographic findings in dogs infected with *D. immitis* and the presence or absence of pulmonary hypertension, as assessed via echocardiography. Specifically, the goal is to determine whether radiographic measurements—such as Vertebral Heart Size (VHS) and the CrPA/R4 and CdPA/R9 ratios—are effective as preliminary screening tools for monitoring pulmonary hypertension.

Radiographically Monitor the Progression of Pulmonary Hypertension Pre- and Post-Adulticide Treatment in Dogs Infected with *D. immitis:* This objective seeks to evaluate the progression of pulmonary hypertension in infected dogs by comparing the Right Pulmonary Artery Distensibility (RPAD) Index before and after adulticide treatment. The aim is to assess the persistence of pulmonary hypertension in hypertensive dogs over time by monitoring changes in VHS, CrPA/R4, and CdPA/R9 ratios, highlighting the importance of radiographic evaluation in the long-term management of the disease.

Identify Radiographic Abnormalities in Cats Infected with Immature Forms of *D. immitis:* This objective focuses on evaluating the radiographic alterations in cats infected with immature forms of *D. immitis*. The emphasis will be on characterizing changes in the pulmonary artery and identifying pulmonary patterns, by using measurements such as the CrPA/R4 and CdPA/R9 ratios, along with measurements of the caudal vena cava (CVC). The goal is to determine the utility of these diagnostic techniques at this stage of infection.

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Association between Thoracic Radiographic Changes and Indicators of Pulmonary Hypertension in Dogs with Heartworm Disease

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Simple Summary: Pulmonary hypertension (PH) is a high-risk condition in dogs with heartworm disease (*Dirofilaria immitis*). Echocardiography is the diagnostic technique of choice to detect PH; however, it is not accessible to all routine clinicians. Therefore, given the importance of this condition during an infection with *D. immitis*, the aim of this study was to evaluate the association of the radiological findings in dogs with heartworm disease and the presence or absence of echocardiographically characterised PH. The results obtained suggest that the evaluation of certain radiographic measures may be useful in the preliminary evaluation of the thoracic radiographs of a dog as a preliminary screening when assessing whether to perform complementary tests to evaluate the presence of PH in dogs with heartworm disease.

Abstract: Pulmonary hypertension (PH) is a consequence of pulmonary endarteritis during infection with *Dirofilaria immitis* in dogs. Echocardiography is the technique of choice but is not always accessible to all clinicians. This study aimed to evaluate the association of the radiological findings in dogs with heartworm disease and the presence or absence of echocardiographically characterised PH. The study included 62 heartworm-infected dogs that underwent thoracic radiographs and echocardiography. The studied dogs showed moderate to severe PH when the Right Pulmonary Artery Distensibility (RPAD) Index was <29.5%. The RPAD Index was used for comparison with thoracic radiographs. The Vertebral Heart Size (VHS), right cranial pulmonary artery passing through the fourth rib in the laterolateral projection (CrPA/R4) ratio, and right caudal pulmonary artery to the ninth rib in the dorsoventral projection (CdPA/R9) ratio showed significant differences between dogs with/without PH ($p < 0.001$). Sensitivity (sen) and specificity (sp) cut-off values were obtained: VHS $\geq$ 9.53 (sen 93.75%, sp 63.33%); CrPA/R4 $\geq$ 1.08 (sen 87.5%, sp 70%); and CdPA/R9 $\geq$ 1.10 (sen 96.88%, sp 76.66%). The CrPA/R4 and CdPA/R9 ratios showed potential as a preliminary screening tool for PH in heartworm-infected dogs, suggesting that they may reliably indicate the presence of PH and guide the decision for further diagnostic testing.

Keywords: vector-borne disease; *Dirofilaria immitis*; pulmonary hypertension; radiographic indexes; image diagnosis; echocardiography; veterinary diagnosis

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Citation: Falcón-Cordón, S.; Falcón-Cordón, Y.; Caro-Vadillo, A.; Costa-Rodríguez, N.; Montoya-Alonso, J.A.; Carretón, E. Association between Thoracic Radiographic Changes and Indicators of Pulmonary Hypertension in Dogs with Heartworm Disease. *Animals* **2024**, *14*, 1900. https://doi.org/10.3390/ani14131900

Academic Editors: Antonio Ortega-Pacheco and Matilde Jimenez-Coello

Received: 21 May 2024
Revised: 18 June 2024
Accepted: 21 June 2024
Published: 27 June 2024

1. Introduction

Pulmonary hypertension (PH) is one of the most common findings in dogs infected by *Dirofilaria immitis* (heartworm disease) as a consequence of the chronic development of proliferative endarteritis within the pulmonary vasculature [1]. The diagnosis of PH is

mainly based on transthoracic Doppler echocardiography, which provides a non-invasive and reliable method for estimating pulmonary arterial pressure since right heart catheterisation, the gold standard for diagnosing PH, is unavailable and unacceptably invasive in compromised patients [2]. However, this method has limitations as the diagnosis is often based on indirect and subjective parameters. In addition, some of these echocardiographic measurements, such as tricuspid valve regurgitation, can be difficult to achieve. On the other hand, other estimators, such as the Right Pulmonary Artery Distensibility Index (RPAD Index), have been shown to be of great help in estimating the presence of PH [3], especially in dogs with heartworm disease [4], which can be useful in cases in which tricuspid regurgitation or pulmonary regurgitation cannot be measured.

The radiographic changes that occur in canine heartworm disease also provide important information. In infected dogs, the main findings are dilatation of the main pulmonary artery and tortuosity of the pulmonary arteries; right ventricular enlargement may also be observed in chronic infestations [5,6]. Furthermore, these signs are associated with the presence of PH in dogs with heartworm disease [7,8]. Thoracic radiography can provide supportive evidence for PH and information on concomitant or causative diseases in an individual dog [2,9], and, unlike echocardiography, this imaging technique is mostly available to the everyday clinician and does not require such specific training or dedicated equipment. Therefore, it would be interesting to perform studies aimed at evaluating its usefulness in detecting the presence of PH in this disease.

In fact, there are studies that have characterised the association of radiological and echocardiographic findings in dogs with PH [10,11], but there are not as many studies performed in dogs with heartworm disease. Given that PH is a common and serious condition in this pathology and given the unique and characteristic changes that pulmonary endarteritis produces in this pathology, this research aims to evaluate the association of the radiological findings in dogs with heartworm disease and the presence or absence of echocardiographically characterised PH.

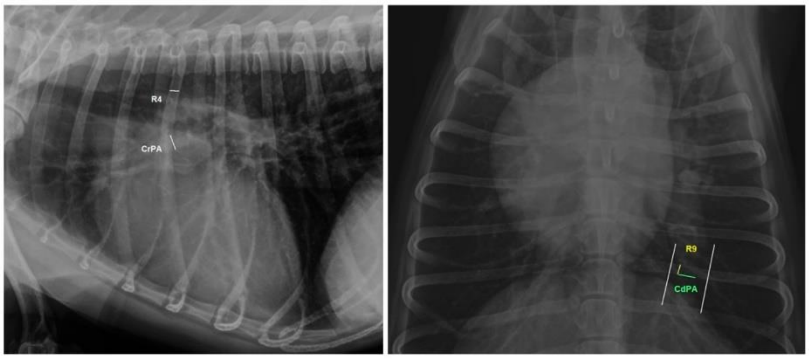
2. Materials and Methods

The study included 62 dogs owned by clients who were brought to the Veterinary Service of the University of Las Palmas of Gran Canaria. The dogs lived in a hyperendemic area for *D. immitis* [12–14]. Inclusion in the study was based on a positive result for circulating *D. immitis* antigens (Urano test Dirofilaria®, Urano Vet SL, Barcelona, Spain). Dogs were also examined for the presence or absence of microfilariae using a modified Knott test. Clinical history and data including age, sex, and breed were recorded for each animal. A complete history and examination were performed on each dog to rule out the presence of other pathologies that might influence the results, and animals with concomitant diseases were excluded from the study.

On the day of diagnosis and the start of treatment (day 0), digital thoracic radiographs were taken using a radiographic unit (RX generator; model: HFQ 300 P, Bennett, NC, USA) at the time of peak inspiration without sedation. The examination protocols (kVP and mAs) were adapted specifically for each dog according to the thoracic thickness of the dog. Views were obtained in its right laterolateral and dorsoventral projections. Vertebral Heart Size (VHS) was measured according to the guidelines of Buchanan and Bücheler 1995 [15]. Although no consideration was given to excluding dogs that might have some type of alteration in the dorsal spine when interpreting VHS, none of the dogs in the present study had such alterations [16].

In addition, the diameter of the right cranial pulmonary artery (CrPA) passing through the fourth rib (R4) in the laterolateral projection and the diameter of R4 at a point just distal to the spine, as well as the distal and left sides of the summation shadow created by the right caudal pulmonary artery (CdPA) with the ninth rib (R9) in the dorsoventral projection, were also measured according to previous guidelines [17]. CrPA/R4 and CdPA/R9 ratios were calculated from these measurements (Figure 1). Measurements were performed using electronic callipers on a DICOM workstation (DAIPACS. 2.71 version). All measurements

were performed by the same technician, blinded to the clinical status of the dogs included in this study.



(**A**) (**B**)

Figure 1. Right laterolateral (**A**) and dorsoventral (**B**) thoracic radiographs illustrating the measurement methods for this study. (**A**) The diameter of the right cranial lobar artery (CrPA) at the level of the fourth rib (R4) and the fourth rib just distal to the spine were measured, and (**B**) the diameter of the caudal lobar artery (CdPA) passing through the ninth rib (R9).

Dogs underwent echocardiographic examination using an ultrasound machine with spectral and colour Doppler and multifrequency probes (5.5–10 MHz) (Logic P5, General Electric, New York, NY, USA). Dogs were placed in the right laterolateral position with the transducer in the third intercostal space. Dogs were conscious and monitored electrocardiographically throughout the study. Six continuous cardiac cycles were recorded for each measurement. All echocardiography exams were performed by the same technician. The presence or absence of PH was determined according to the American College of Veterinary Internal Medicine (ACVIM) guidelines [9]. Of all the echocardiographic indices studied, the determination of the Right Pulmonary Artery Distensibility Index (RPAD Index) was used in this study for comparison with thoracic radiographs as all dogs showed higher likelihood of moderate to severe PH when the RPAD Index was <29.5%, as previously described and validated in dogs with heartworm disease [3,4,18].

In addition, other echocardiographic findings were used to estimate worm burden [19], and a score of 1 to 4 was assigned from low to high worm burden as follows: (1) no worms visualised, (2) few worm echoes in the distal part of the right pulmonary artery, (3) worm echoes occupying the right pulmonary artery and extending to the main pulmonary artery, and (4) worm echoes occupying the entire right pulmonary artery and the main pulmonary artery to the level of the pulmonary valve. Scores of 1 and 2 corresponded to low parasite burden, and scores of 3 and 4 corresponded to high burden.

The data were analysed using the SPSS Base 29.0 software for Windows (SPSS Inc./IBM, Chicago, IL, USA). A Shapiro–Wilk test was performed to verify the normal distribution of the data. Continuous variables were expressed as median ± standard deviation. Qualitative variables were expressed as percentage. The chi-squared test or Fisher's exact test was used to assess the association between categorical variables. In all cases, a p value < 0.05 was determined as significant. The results of the statistical procedures were also graphed by scatter plot. A simple linear regression was performed between the RPAD Index values and the other variables studied (VHS, CrPA/R4, and CdPA/R9 ratios) to identify the best one-variable model, and a regression analysis of all subsets was performed with a maximum improvement of R^2 as a selection criterion. Receiver operator characteristic curve (ROC) analyses were performed to determine the optimal cut-off values for the prediction of the RPAD Index being <29.5% (moderate or severe hypertension). For all results, $p < 0.05$ was considered statistically significant. Furthermore, Cohen's D was employed to interpret

the differential magnitude between the studied statistical groups considering a statistical difference with values > 0.70 for this study.

All the owners provided their consent to participate in this study, which was carried out in accordance with the current European legislation on animal protection.

3. Results

Of the studied dogs, 27 were male and 35 were female, with the ages ranging from 1.5 to 12 years (mean: 5.25 years). Based on breed, 37 were mixed-breed dogs and 25 were pure-bred dogs. PH was present in 32 dogs (51.6%), with a mean RPAD Index of 29.1%. Microfilaremia was present in 40.3% of the dogs. The parasite burden was low in 87.1% of the dogs and high in 12.9% of them.

The results showed significant differences between the body weight and worm burden ($p = 0.023$), with the mean weight being 18.3 ± 10.3 kg for the dogs with a low worm burden and 11.1 ± 7.8 kg for the dogs with a high worm burden. Additionally, the dogs with PH were significantly older ($p = 0.021$).

The VHS showed a mean value of 10.1 ± 0.8 for all the studied dogs, with significant differences observed when differentiating based on the presence or absence of PH (10.41 ± 0.81 vs. 9.72 ± 0.81, respectively) ($p < 0.001$). There were no statistically significant differences in the VHS values based on the microfilaremia, parasite load, age, or sex.

The results for the CrPA/R4 and CdPA/R9 ratios were 1.21 ± 0.39 and 1.4 ± 0.5, respectively. Significant differences were observed between the groups for the CrPA/R4 ratio (1.37 ± 0.44 vs. 1.01 ± 0.23, respectively) ($p < 0.001$) and for the CdPA/R9 ratio (1.63 ± 0.56 vs. 1.16 ± 0.27, respectively) ($p < 0.001$) (Table 1). Statistically significant differences were also found for the CdPA/R9 ratio in relation to the presence or absence of microfilaremia (1.45 ± 0.35 vs. 1.37 ± 0.58, respectively) ($p = 0.031$). No significant differences were found for the rest of the studied parameters (parasite load, age, or sex).

Table 1. Correlation coefficients for all studied radiographic parameters.

Correlation	Coefficient	Interpretation
IDAPD-VHS	−0.4776417 ***	Moderate negative correlation
IDAPD-CRPA/R4	−0.4344274 ***	Moderate negative correlation
IDAPD-CDPA/R9	−0.53781329 ***	Moderate negative correlation

*** Correlation is significant at 0.5% ($p < 0.005$).

The Pearson correlation model was used to determine whether there was a correlation between the presence of PH, based on the RPAD Index, and the studied radiographic parameters (VHS, CrPA/R4, and CdPA/R9 ratios). The correlations obtained for all three were moderately negative, indicating that, as the RPAD Index decreased, the other parameters increased. Furthermore, the results for all three correlations were statistically significant ($p < 0.005$) (Table 1).

In this study, regression analysis was performed to determine the area under the curve (AUC), coefficient of determination (R^2), and the specificity and sensitivity of the radiographic indicators (VHS, CrPA/R4, and CdPA/R9 ratios). In addition, cut-off values were established for each of these parameters.

The CdPA/R9 ratio model proved to be the most effective in explaining the variability of the dependent variable (RPAD Index), with an R^2 of 0.976, followed by the CrPA/R4 ratio and VHS. This indicated that CdPA/R9 had a superior ability to model the influence of the independent variables on the RPAD Index.

AUC values were calculated for each radiographic indicator and showed that all the values were above 0.5 but below 1. This suggested that the models provided a more accurate classification than would be achieved by chance, enabling the correct prediction of positive and negative cases. Specifically, the AUC for VHS was 0.78, indicating good discriminatory ability, with a 78% probability of distinguishing between the positive and negative cases.

Similarly, the CrPA/R4 ratio showed an AUC of 0.77, and the CdPA/R9 ratio, the highest, reached an AUC of 0.82, highlighting its superior performance in model discrimination.

For VHS, a cut-off of 9.53 or higher resulted in a sensitivity of 93.75% and a specificity of 63.33%. This indicated a high ability to detect true positive cases, but with a moderate false positive rate. For the CrPA/R4 ratio, a cut-off of 1.08 or higher yielded a sensitivity of 87.5% and a specificity of 70%, providing a reasonable balance between detecting positive cases and minimising false positives. Finally, the cut-off for the CdPA/R9 ratio was set at 1.10 or higher, providing a sensitivity of 96.88% and a specificity of 76.66%, making it the most efficient of the three in terms of correctly identifying both positive and negative cases (Table 2).

Table 2. Results of simple regression analyses for the prediction of PH using the Right Pulmonary Artery Distensibility Index (RPADI < 29.5%). R^2 (coefficient of determination); AUC (area under receiver operating characteristic curve); VHS (Vertebral Heart Size); CrPA/R4 (right cranial pulmonary artery passing through the fourth rib in the laterolateral projection ratio); CdPA/R9 (right caudal pulmonary artery to the ninth rib in the dorsoventral projection ratio).

Parameter	R^2	AUC	Cut-Off Value	Sensitivity	Specificity
VHS	0.853	0.78	≥9.53	93.75%	63.33%
CRPA/R4	0.959	0.77	≥1.08	87.5%	70%
CDPA/R9	0.976	0.82	≥1.10	96.88%	76.66%

In addition, Cohen's D, a standardised measure of effect size, was used to interpret the magnitude of the differences between the statistical groups studied. For VHS, the calculated Cohen's D value of 22.11 indicated a substantial difference between the dogs with the presence of pulmonary hypertension and those in absence. This extremely large effect size suggests a clinically meaningful distinction between the two groups in terms of the VHS measurements. Similarly, for the CRPA/R4 ratio, the Cohen's D value of 4.31 indicated a significant difference between the groups. Although this is slightly smaller than the effect size for VHS, it is still a large effect size, indicating a notable difference in the CRPA/R4 ratio between the two groups. In the case of the CDPA/R9 ratio, the Cohen's D value of 0.702, while indicating a smaller effect size compared to VHS and the CDPA/R4 ratio, still reflects a moderate difference between the dogs without PH and the group with the presence of PH. This suggests that, although the effect size was smaller, the CDPA/R9 ratio remains a relevant and potentially informative parameter to discriminate between these groups, underlining its importance in the assessment of PH (Table 3).

Table 3. Cohen's D for the three studied parameters. VHS (Vertebral Heart Size); CrPA/R4 (right cranial pulmonary artery passing through the fourth rib in the laterolateral projection ratio); CdPA/R9 (right caudal pulmonary artery to the ninth rib in the dorsoventral projection ratio).

Measure	Cohen's D	Interpretation
VHS	22.1	Large effect
CrPA/R4	4.31	Large effect
CdPA/R9	0.702	Moderate effect

Cohen's D: d = 0.2 small effect; d = 0.5 moderate effect; d = 0.8 large effect.

4. Discussion

Pulmonary hypertension is caused by the presence of adult *D. immitis* parasites as a result of the lesions they cause in the pulmonary arteries from the early stages of parasitism. These lesions lead to proliferative endarteritis, resulting in the enlargement, tortuosity, and loss of elasticity of the pulmonary vasculature [1]. PH is a common phenomenon in this pathology, severe and apparently irreversible in most cases [20,21], so its study and early detection should be a priority.

Echocardiography is the method of choice to detect this condition, but this technique is often inaccessible to clinical veterinarians, either due to a lack of knowledge or equipment, or to animal owners due to financial constraints. However, radiology is a diagnostic imaging technique widely used in all veterinary clinics that is less technically demanding and more affordable [22]. Therefore, in this study, the authors aimed to evaluate its usefulness as a first approximation imaging tool to determine the presence or absence of PH in dogs with heartworm disease.

The results showed significant differences between the VHS indices and the CrPA/R4 and CdPA/R9 ratios in animals with PH compared with those without PH in dogs with heartworm disease. Previous studies have demonstrated radiological changes in dogs with PH caused by several pathologies, mainly cardiomegaly, with other findings described as infiltration of the lung parenchyma and enlargement of the pulmonary arteries [10,23]. In addition, the ECVIM guidelines recommend that echocardiography be performed when a dog has radiological changes consistent with PH [9]. Indeed, thoracic radiography is a useful technique for detecting cardiopulmonary abnormalities in dogs with *D. immitis*, with the enlargement of the right ventricle, the main pulmonary artery, and the right lobar pulmonary artery being the most commonly reported abnormalities [24–26]. However, no studies were found on the use of radiological changes objectively as an estimator of PH, so the results of this study validate the clinical usefulness of radiographs in dogs with heartworms to estimate the presence of PH.

The results obtained showed that the VHS was increased in the group with PH compared to the group without PH. Other authors have reported increased cardiac silhouettes on thoracic radiographs in dogs with PH, especially when symptomatic [25,27]. The cut-off value of 9.53 provides high sensitivity but low specificity for the detection of PH, which is consistent as the value of 9.53 is within the normal range for healthy dogs [15]. Therefore, although statistically there are differences between VHS as a function of the presence or absence of PH, and there is a significant correlation between the RPAD Index and VHS, it could not be considered an adequate value to determine the presence of this condition with the results of this study. In dogs with heartworm disease, cardiomegaly occurs only in the final stages of the disease and in dogs with severe PH, whereas this study included dogs with PH considered to be moderate to severe.

The comparison between the pulmonary arteries and the rib is a useful and common criterion to assess the pulmonary vasculature in dogs, as pointed out by previous authors [17,28], also in canine heartworm disease [24,25]. In *D. immitis* infection, there is enlargement, dilation, and increased tortuosity of the pulmonary arteries as a result of endarteritis, leading to PH. However, whether these vascular changes can act as predictors of PH in infected dogs has never been investigated. The results showed that both ratios showed significant differences between the presence/absence of PH. Furthermore, there was a correlation between both ratios and the RPAD Index, especially for the CdPA/R9 ratio. The cut-off values for the CrPA/R4 ($\geq$1.08) and CdPA/R9 ($\geq$1.10) ratios showed good sensitivity, excellent in the case of the CdPA/R9 ratio, with good specificity, indicating that these parameters could be used in the radiological evaluation of dogs with heartworm disease as a preliminary screening to determine the need for further testing for the presence of PH. These cut-off values are different from the reference values established by previous authors for the CrPA/R4 ratio < 1.2 [28] and CdPA/R9 of 1 for healthy dogs [29,30], or those described to differentiate dogs with mitral regurgitation from healthy dogs (CrPA/R4 ($\geq$0.95) and CdPA/R9 $\geq$ 1.32) [17].

Other authors have already demonstrated the usefulness of these radiological indices to detect PH caused by other thoracic pathologies, such as Oui et al. (2015) [17], who used these indices, among others, to differentiate between dogs with mitral regurgitation and healthy dogs. In addition, other authors have demonstrated the usefulness of other similar radiological indicators to predict the presence of PH caused by different pathologies, such as the ratio of the area of the pulmonary artery crossing the ninth rib to the area of the ninth thoracic vertebra (areaPA/areaT9), the ratio of the width of the pulmonary artery crossing

the ninth rib to the width of the ninth thoracic vertebra (widthPA/widthT9), or the caudal pulmonary artery to vein ratio [11,31].

As a limitation of the study, it must be remembered that, in veterinary medicine, the measurement of PH is based on indirect echocardiographic measurements and that they have not been confirmed with direct measurements through right heart catheterisation.

5. Conclusions

In conclusion, an increase in the cardiac silhouette does not appear to be useful in assessing the presence or absence of PH in dogs with heartworm disease. However, the results obtained for the CrPA/R4 and CdPA/R9 ratios seem to show cut-off values with quite acceptable sensitivity and specificity, which could suggest the evaluation of these ratios when carrying out a preliminary evaluation of the thoracic radiographs of a dog as a preliminary screening when assessing whether to perform complementary tests to evaluate the presence of PH. Moreover, additional studies with a larger number of animals, to enable a more robust statistical analysis, are necessary to further evaluate these radiological indicators.

Author Contributions: J.A.M.-A. and E.C. designed the study. S.F.-C., Y.F.-C., and E.C. wrote the manuscript. S.F.-C., Y.F.-C., A.C.-V., and N.C.-R. performed the fieldwork, collected the data, and performed the experiments. All authors participated in the discussion of the results, corrected, read, and approved the final manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: The presented study was supported by own funds from the Internal Medicine Service FULP/ULPGC (SD-240/030/0026). N.C.-R. was supported by the "Grants for the predoctoral training program for researchers" programme of the Government of the Canary Islands (TESIS2021010010).

Institutional Review Board Statement: Ethical review and approval were not required for the animals in this study. All radiographs and echocardiographic measures were routinely collected for prescribed diagnostic purposes or official monitoring studies and subsequently made available to this study. All the dog owners were informed about the present study and consented to participate. The study was carried out in accordance with the current Spanish and European legislation on animal protection (Spanish Royal Decree 53/2013 and 2010/63/UE Directive).

Informed Consent Statement: Written informed consent has been obtained from the owner of the animals involved in this study.

Data Availability Statement: All data generated or analysed during this study are included in this article. The datasets used and/or analysed during the present study are available from the corresponding author upon reasonable request.

Acknowledgments: The authors would like to thank Uranovet for kindly providing the Urano test Dirofilaria®. Also, they would like to thank the Hospital Clínico Veterinario of the Universidad de Las Palmas de Gran Canaria (ULPGC) for their support.

Conflicts of Interest: The authors declare no conflicts of interest.

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Assessment of Thoracic Radiographic Alterations in Dogs with Heartworm and Their Correlation with Pulmonary Hypertension, Pre- and Post-Adulticide Treatment

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Simple Summary: Pulmonary hypertension (PH) is a common and severe complication in dogs infected with *Dirofilaria immitis*, often persisting even after treatment. This study aimed to evaluate the progression of PH in dogs by assessing changes in radiographic parameters and the Right Pulmonary Artery Distensibility (RPAD) Index before and after treatment. Parameters were measured on the day of diagnosis (Day 0), at discharge (Day 90), and six months post-discharge (Day 270). The results indicated that in non-hypertensive dogs, the RPAD Index significantly improved following treatment. In contrast, hypertensive dogs exhibited a persistently low RPAD Index throughout the study, indicating ongoing PH. Additionally, hypertensive dogs showed consistently elevated VHS, CrPA/R4, and CdPA/R9 ratios compared to non-hypertensive dogs. These findings underscore the persistence of PH despite treatment, suggesting that regular radiographic monitoring of VHS, CrPA/R4, and CdPA/R9 ratios is crucial for assessing and managing long-term outcomes in dogs with heartworm disease.

Abstract: Pulmonary hypertension (PH) is a prevalent and severe complication in dogs infected with *Dirofilaria immitis*. This study aimed to elucidate the progression of PH by analyzing radiographic parameters and the Right Pulmonary Artery Distensibility (RPAD) Index at three key time points: diagnosis (day 0), discharge (day 90), and six months post-discharge (day 270). Fifty-two heartworm-infected dogs were divided into two groups: non-hypertensive and hypertensive. Radiographic measurements, including Vertebral Heart Size (VHS), CrPA/R4 ratio, and CdPA/R9 ratio, along with the RPAD Index, were assessed on Days 0, 90, and 270. Results indicated that, in Group A, the RPAD Index improved significantly from 42% on Day 0 to 43.16% on Day 90, with no significant change by Day 270 (42%). In contrast, hypertensive dogs exhibited a persistently low RPAD Index, averaging 17% throughout this study ($p < 0.001$). Radiographic parameters in hypertensive dogs showed continuous elevation compared to non-hypertensive dogs, with significant increases in VHS, CrPA/R4, and CdPA/R9 ratios on day 270 compared to day 0 ($p < 0.05$). The results confirmed that PH persisted in dogs with *D. immitis* after adulticide treatment, highlighting the importance of regular radiographic monitoring for assessing and managing long-term outcomes in dogs with PH during and after adulticide treatment. Continuous surveillance is thus essential for the effective post-treatment management of PH in dogs.

Keywords: vector-borne disease; *Dirofilaria immitis*; pulmonary hypertension; radiographic indexes; image diagnosis; echocardiography; veterinary diagnosis; dogs

Citation: Falcón-Cordón, S.; Falcón-Cordón, Y.; Costa-Rodríguez, N.; Matos, J.I.; Montoya-Alonso, J.A.; Carretón, E. Assessment of Thoracic Radiographic Alterations in Dogs with Heartworm and Their Correlation with Pulmonary Hypertension, Pre- and Post-Adulticide Treatment. *Animals* **2024**, *14*, 2551. https://doi.org/10.3390/ani14172551

Academic Editors: Antonio Ortega-Pacheco and Matilde Jimenez-Coello

Received: 25 July 2024
Revised: 26 August 2024
Accepted: 31 August 2024
Published: 2 September 2024

1. Introduction

Heartworm disease is caused by the nematode *Dirofilaria immitis*, with adult worms lodging in the pulmonary arteries and right ventricle and mainly affecting domestic and wild carnivores [1]. Pulmonary arterial hypertension (PH) is a frequent and generally severe condition in infected dogs; it is caused primarily by proliferative intimal changes driving irreversible structural damage to the vasculature, inflammation, loss of elasticity, and occlusion of the vascular lumen, leading to persistent PH [2,3]. In addition, embolization of dead heartworms contributes to the development and perpetuation of PH [1,4].

Previous authors agreed that severity and chronicity of pulmonary endarteritis can be assessed through the determination of PH [5–8]. To this aim, the use of echocardiography can provide valuable information for diagnosing PH based on indirect measurements, since right heart catheterization, which is the gold standard for diagnosing PH, is unacceptably invasive in routine procedures or in compromised patients [9,10].

Thoracic radiography is a valuable tool for identifying concurrent or underlying diseases in an individual patient and can also provide evidence supporting the presence of PH [9,11]. This imaging modality has the advantage that it is widely accessible for clinicians on a daily basis. Indeed, there are studies that have characterized the association of radiological and echocardiographic findings in dogs with PH [12,13]. In dogs with heartworm, thoracic radiographs may reveal right ventricular enlargement, main pulmonary artery dilation, and pulmonary artery tortuosity, although these findings are not specific [3,14]. Moreover, chest radiographs are useful in helping to estimate the severity and chronicity of heartworm disease [15]. More recently, a study in dogs with heartworm reported that evaluating certain thoracic radiographic measurements can serve as an objective preliminary screening when assessing whether complementary tests should be performed to evaluate the presence of PH [16].

Previous studies have shown that PH persisted in dogs after heartworm adulticide treatment for a minimum of 10 months following the completion of heartworm removal. The authors suggested that endarteritis may not be reversible after parasite elimination, highlighting the need for continued monitoring for PH after completion of adulticide treatment [8,17,18]. However, there is currently no research associating specific thoracic radiographic findings with the severity of PH in *D. immitis*-infected dogs and their changes following adulticide treatment. Therefore, the objective of this study was to determine if specific radiographic findings are linked to the severity of PH in naturally infected dogs before and after adulticide treatment.

2. Materials and Methods

2.1. Enrollment and Treatment of the Dogs

This research included 52 dogs owned by clients, which were taken to the Veterinary Medicine Service of the Veterinary Teaching Hospital of the University of Las Palmas de Gran Canaria (Spain). These dogs resided in an area with a high prevalence of heartworm [19–21] and were selected for this study based on a positive test result for circulating *D. immitis* antigens (Urano test Dirofilaria®, Urano Vet SL, Barcelona, Spain). Clinical history and information of each animal, such as age, sex, and breed, were recorded. A thorough medical history and examination were conducted for each dog to rule out the presence of other diseases that could impact the results. Any animal that was given medication for cardiovascular conditions was not included in this research. Likewise, dogs showing symptoms of heart disease (such as valvular heart disease, cardiomyopathy, and congenital defects) were not included in this research. Diagnostic imaging tests (thoracic radiography and echocardiography), as well as physical examination, clinical history, and anamnesis, were also used to rule out other coexisting cardiorespiratory conditions.

The infected canines underwent adulticide treatment as per the treatment protocol advised by the international Heartworm Societies, incorporating the recently published modifications [22–24]. To summarize, upon diagnosis on day 0, the dog started doxycycline administration (10 mg/kg BID) for 4 weeks for the treatment of the endosymbiont bacteria

Wolbachia pipientis, along with monthly oral tablets for heartworm prevention containing ivermectin (≥6 mcg/kg) and pyrantel pamoate (≥5 mg/kg). On days 30, 60, and 61, the dog received intramuscular melarsomine doses (2.5 mg/kg). A follow-up examination on day 90 determined discharge eligibility based on the absence of abnormalities (adult parasites in echocardiography, radiographic irregularities, or cardiorespiratory symptoms). After 6 months from discharge, on day 270, adulticidal efficacy was confirmed through an antigen detection test. It was advised to restrict exercise during the treatment period, especially from the first melarsomine dose until discharge.

2.2. Radiograph and Ultrasound Evaluations

On day 0 (diagnosis), day 90 (discharge), and 270 (6 months after discharge), thoracic radiographs were digitally captured using an RX generator (HFQ 300 P, Bennett, NC, USA) during peak inspiration, without sedation. The radiographic parameters (kVP and mAs) were adjusted individually for each dog based on thoracic thickness. Both right laterolateral and dorsoventral views were taken. Vertebral Heart Size (VHS) was determined following the guidelines previously established by Buchanan and Bücheler [25]. While potential dorsal spine alterations were not a basis for exclusion in VHS interpretation, none of the dogs in this study exhibited such alterations [26]. Furthermore, the CrPA/R4 ratio was calculated based on the measurement of the diameter of the right cranial pulmonary artery (CrPA) passing through the fourth rib (R4) in the laterolateral view, as well as the diameter of R4 just distal to the spine [16,27]. Finally, the CdPA/R9 ratio was determined in the dorsoventral view by the measurement of the diameter of the right caudal pulmonary artery (CdPA) overlapping the ninth rib (R9), in accordance with established guidelines and previous studies [16,27]. All measurements were conducted using electronic callipers on a DICOM workstation (DAIPACS, version 2.71) by a researcher who was unaware of the clinical status of the dogs involved in this study.

The canines also underwent echocardiographic evaluations on days 0, 90, and 270 utilizing an ultrasound device equipped with spectral and color Doppler as well as multifrequency probes (5.5–10 MHz) (Logic P5, General Electric, New York, NY, USA) to determine the presence or absence of PH, in accordance with the guidelines set by the American College of Veterinary Internal Medicine (ACVIM) [11]. The dogs were positioned in right laterolateral recumbence with the transducer placed in the third intercostal space. They remained conscious and were continuously monitored electrocardiographically. Each measurement involved recording six consecutive cardiac cycles, and all echocardiographic assessments were performed by the same researcher. To the aims of this study, the Right Pulmonary Artery Distensibility Index (RPAD Index) was utilized for comparison with thoracic radiographic measurements, with dogs being more likely to exhibit moderate to severe PH if their RPAD Index was <29.5%, a criterion that has been described and validated in dogs affected by heartworm disease [28–30]. The dogs were divided into 2 groups: Group A included dogs without PH, and Group B included dogs with PH. All dogs with PH showed a RPAD Index < 29.5%, and all dogs without PH showed a RPAD Index > 29.5%.

2.3. Statistical Analysis

The data were analyzed using the SPSS Base 29.0 software for Windows (SPSS Inc./IBM, Chicago, IL, USA). A Shapiro–Wilk test was performed to verify the normal distribution of the data. Additionally, the Siegel–Tukey test was performed to verify the variability of variances between groups. Continuous variables were expressed as mean ± standard deviation. Qualitative variables are expressed as percentages. The non-parametric test of Wilcoxon was used to determine the differences in the different stages (day 0, 90, and 270). In addition, a U–Mann–Whitney test was used to determine differences between dogs with and without PH. In all cases, a p-value < 0.05 was determined as significant. Furthermore, Cohen's D was employed to interpret the differential mag-

nitude between the studied statistical groups. Considering a statistical difference with values > 0.70 for this study.

All dog owners gave their approval for their pets to take part in this research, and an informed consent was specifically signed for this purpose. This study was carried out in compliance with the existing animal welfare laws in Europe.

3. Results

Of the dogs studied, 57.7% (30/52) were males and 42.3% (22/52) were females, ranging in age from 1 to 14.5 years (mean: 5.5 ± 0.4 years). Regarding breed, 75% (39/52) were mixed-breed dogs, and 25% (13/52) were purebred. Of the latter, PH was present in the following breeds: Labrador Retriever (n = 5), American Pit Bull Terrier (n = 1), and Rat Terrier (n = 1). On the other hand, PH was absent in the following purebred dogs: Garafian Shepherd (n = 2), Canarian Mastiff (n = 1), Spanish Water Dog (n = 1), Miniature Pinscher (n = 1), and Rat Terrier (n = 1). Regarding sex, 42.3% (22/52) were females and 57.7% (30/52) were males. In addition, the mean weight of the animals included in this study was 15.33 ± 1.35 kg. On day 0, 40.4% (21/52) of the dogs had PH with a mean RPAD Index of 17%, whereas 59.6% (31/52) were normotensive with a mean RPAD Index of 42%.

Symptoms were observed in 19.35% of dogs without PH, whereas all dogs diagnosed with PH exhibited symptoms, with cough and dyspnea being the most common (Table 1).

Table 1. Distribution of symptoms and frequency by groups. Group A: dogs without pulmonary hypertension. Group B: dogs with pulmonary hypertension.

Groups	Symptoms	Percentage
Group A	Asymptomatic	80.65% (25/31)
	Cough	19.35% (6/31)
Group B	Asymptomatic	0% (0/21)
	Cough	95.24% (20/21)
	Dyspnea	57.14 (12/21)
	Weight loss	4.76% (1/21)

The Wilcoxon-signed rank test was used to compare the differences in scores between patients on days 0, 90, and 270. For the RPAD Index, only significant differences were observed between days 0 and 90 (p = 0.011). For VHS, statistically significant differences were observed between day 0 and day 90 (p = 0.001) and between day 0 and day 270 (p = 0.000). However, no statistically significant differences were observed between the measurements at the three time points for the CrPA/R4 and CdPA/R9 ratios (Table 2).

As described in the Section 2, the dogs were further divided into two groups: Group A included dogs without PH (n = 31), and Group B included dogs with PH (n = 21).

The results showed that RPAD Index values were significantly lower in the group of dogs with PH at all time points ($p = 2.1218 \times 10^{-14}$ for day 0, $p = 2.1218 \times 10^{-14}$ for day 90, and $p = 1.4216 \times 10^{-12}$ for day 270). In addition, while the RPAD Index increased significantly between days 0 and 90 in dogs from group A, the RPAD Index did not change significantly throughout the treatment in dogs with PH (Table 3). Additionally, a dog in group A experienced a decline in pulmonary arterial values, leading to the end of treatment with the presence of PH and an RPAD Index of 22%.

Table 2. Results of radiographic measures in all studied dogs in different time points. RPAD Index (Right Pulmonary Artery Distensibility Index); VHS (Vertebral Heart Size); CrPA/R4 (right cranial pulmonary artery passing through the fourth rib in the laterolateral projection ratio); CdPA/R9 (right caudal pulmonary artery to the ninth rib in the dorsoventral projection ratio). (*): $p < 0.05$.

Measure	Time Point	*p*-Value
RPAD Index	Day 0–Day 90	0.01108686 *
	Day 0–Day 270	0.37772975
	Day 90–Day 270	0.14238119
VHS	Day 0–Day 90	0.00137051 *
	Day 0–Day 270	0.00027207 *
	Day 90–Day 270	0.05733536
CrPA/R4	Day 0–Day 90	0.16833147
	Day 0–Day 270	0.2023426
	Day 90–Day 270	0.36260403
CdPA/R9	Day 0–Day 90	0.16833147
	Day 0–Day 270	0.22349953
	Day 90–Day 270	0.18534784

Table 3. Results of the Wilcoxon test for all measures by time points and groups of studied dogs. RPAD Index (Right Pulmonary Artery Distensibility Index); VHS (Vertebral Heart Size); CrPA/R4 (right cranial pulmonary artery passing through the fourth rib in the laterolateral projection ratio); CdPA/R9 (right caudal pulmonary artery to the ninth rib in the dorsoventral projection ratio). Group A: dogs without pulmonary hypertension. Group B: dogs with pulmonary hypertension. Results are displayed as mean ± standard deviation. NS: No significant differences were found between time points. (*): $p < 0.05$.

Measure	Group	Day 0	Day 90	Day 270	Time Points with Significant Differences between Measures	*p* Value
RPAD Index	Group A	42% ± 0.06	43.16% ± 0.07	42% ± 0.07	Day 0–Day 90	0.043641 *
	Group B	17% ± 0.11	17.96% ± 0.11	17.84% ± 0.11	NS	>0.05
VHS	Group A	9.85 ± 0.74	9.92 ± 0.68	9.98 ± 0.72	Day 0–Day 270	0.01313415 *
	Group B	10.29 ± 0.74	10.43 ± 0.76	10.48 ± 0.76	Day 0–Day 90 Day 0–Day 270	0.01844421 * 0.01313415 *
CrPA/R4	Group A	1.08 ± 0.16	1.06 ± 0.18	1.06 ± 0.16	NS	>0.05
	Group B	1.54 ± 0.36	1.59 ± 0.41	1.60 ± 0.47	Day 0–Day 90 Day 0–Day 270	0.01448255 * 0.04484749 *
CdPA/R9	Group A	1.14 ± 0.15	1.13 ± 0.16	1.12 ± 0.15	NS	>0.05
	Group B	1.68 ± 0.38	1.77 ± 0.39	1.79 ± 0.39	Day 0–Day 90 Day 0–Day 270	0.02499993 * 0.01999474 *

Regarding the radiographic indices, the mean VHS for each group on days 0, 90, and 270 are shown in Table 3. These results indicated that the mean VHS was significantly higher in Group B than in Group A at all time points ($p = 0.03645206$ for day 0, $p = 0.0131386$ for day 90, and $p = 0.01618378$ for day 270). In addition, a significant increase in VHS was observed between days 0 and 270 for Group A dogs and between days 0–90 and days 0–270 for Group B dogs. These results show that VHS tended to increase over time from day 0 to 6 months after treatment, particularly in the group of hypertensive dogs. When compared with the established reference values [25], the number of dogs in Group B exceeding the reference range increased towards the end of this study, whereas the number of dogs in Group A exceeding the reference value remained stable throughout this study (Table 4).

Table 4. Results for Vertebral Heart Score (VHS) results are distributed by time points in the studied dogs. Group A: dogs without pulmonary hypertension. Group B: dogs with pulmonary hypertension. Results are displayed as mean ± standard deviation.

	Groups	VHS	Dogs above Reference Value (≥10.5) [25]
Day 0	GROUP A	9.85 ± 0.74	12.90% (4/31)
	GROUP B	10.29 ± 0.74	28.57% (6/21)
Day 90	GROUP A	9.92 ± 0.68	12.90% (4/31)
	GROUP B	10.43 ± 0.76	42.86% (9/21)
Day 270	GROUP A	9.98 ± 0.72	12.90% (4/31)
	GROUP B	10.48 ± 0.76	47.62% (10/21)

The CrPA/R4 and CdPA/R9 ratios were significantly higher in Group B dogs at all time points (CrPA/R4: $p = 2.5007 \times 10^{-8}$ for day 0, $p = 2.5007 \times 10^{-8}$ for day 90, and $p = 7.5505 \times 10^{-8}$ for day 270; CdPA/R9: $p = 2.0504 \times 10^{-9}$ for day 0, $p = 5.7586 \times 10^{-11}$ for day 90, and $p = 1.4513 \times 10^{-11}$ for day 270). In addition, the CrPA/R4 and CdPA/R9 ratios remained constant without significant variation throughout the treatment in dogs from Group A; however, significant modifications were observed in dogs from Group B during adulticide treatment, which were statistically significant between days 0 and 90 and tended to increase during this study (Table 3).

Cohen's D, a standardized measure of effect size, was used to interpret the magnitude of differences between the statistical groups studied. For VHS, the calculated Cohen's D value was between 20.99 and 21.29, indicating a substantial difference between normotensive and hypertensive dogs. This extremely large effect size suggests a clinically meaningful distinction between the two groups in terms of VHS measurements. Similarly, for the CrPA/R4 ratio, the Cohen's D value was between 2.87 and 2.89, indicating a significant difference between the normotensive and hypertensive groups. Although this was slightly smaller than the effect size for VHS and the CdPA/R9 ratio, it was still a large effect size, indicating a notable difference in the CRPA/R4 ratio between the two groups. In the case of the CdPA/R9 ratio, the Cohen's D value was between 3.08 and 3.15, while indicating a smaller effect size compared to VHS, still reflected a moderate difference between the normotensive and hypertensive groups, remaining a relevant and potentially informative parameter to discriminate between these groups, underscoring its importance in the assessment of PH (Table 5).

Table 5. Cohen's D for the studied parameters. RPAD Index (Right Pulmonary Artery Distensibility Index); VHS (Vertebral Heart Size); CrPA/R4 (right cranial pulmonary artery passing through the fourth rib in the laterolateral projection ratio); CdPA/R9 (right caudal pulmonary artery to the ninth rib in the dorsoventral projection ratio).

Measure	Cohen's D	Value	Interpretation
VHS	Day 0	20.9929921	Large effect
	Day 90	21.1714818	Large effect
	Day 270	21.2935957	Large effect
CrPA/R4	Day 0	2.86616835	Large effect
	Day 90	2.88762566	Large effect
	Day 270	2.84047934	Large effect
CdPA/R9	Day 0	3.07601672	Large effect
	Day 90	3.15304145	Large effect
	Day 270	3.11923901	Large effect

Cohen's D: d = 0.2 small effect, d = 0.5 moderate effect; d = 0.8 large effect.

4. Discussion

Several studies have suggested that adult *D. immitis* parasites begin to produce lesions once they reach the pulmonary arteries, resulting in proliferative endarteritis that chronically leads to PH and heart failure [3]. PH is therefore a lesion associated with proliferative endarteritis and is consequently common in this disease.

The determination of PH can be indirect, as its direct estimation by right heart catheterization is neither clinically nor practically feasible [31]. Therefore, echocardiography is considered the best method of measurement. Furthermore, previous studies have shown that thoracic radiographs are useful in determining the presence and severity of lesions associated with PH, both in heartworm infection and in other cardiopulmonary pathologies [2,32,33], and some studies have even characterized the association between some radiological changes and echocardiographic findings in dogs with PH [12,13]. As in the present study, these authors reported the presence of cardiomegaly and enlarged pulmonary arteries, among other findings. Recently, a study showed that the assessment of VHS, CrPA/R4, and CdPA/R9 ratios may be used objectively in the preliminary evaluation of chest radiographs of dogs with PH as a screening tool when deciding whether to perform additional tests to assess the presence of PH [16]. The results of the present study confirmed the findings of the previous study, as statistically significant increases in the parameters evaluated (VHS, CrPA/R4, and CdPA/R9 ratios) were observed at all time points between dogs with and without PH.

Furthermore, other previous studies using echocardiography and specific serum biomarkers (i.e., endothelin-1 and acute phase proteins) have shown that PH persisted in dogs after the end of adulticide treatment, at least 10 months after the last dose of melarsomine, reporting that proliferative endarteritis may not be reversible and may persist even after the elimination of adult parasites, thus being a chronic problem that may affect the quality of life and life expectancy of the dog [8,17,18]. Therefore, its study and early detection should be a priority. The results of this study would add to this evidence by demonstrating the persistence of PH, in this case by means of a thoracic radiographic study.

Regarding radiographic indices, the results show an increase in VHS in dogs with PH throughout this study. These results are similar to those reported by other authors who have suggested that heartworm-infected dogs often have an enlarged cardiac silhouette on thoracic radiographs, which is a reliable parameter to discriminate severe PH from non-pulmonary hypertensive dogs [34]. When compared to the reference values [25], 28.6% of dogs with PH had a VHS above the reference value on day 0, rising to 47.6% by the end of this study. Chronic persistence of PH eventually leads to right-sided heart disease, and the observed increase in cardiac silhouette in dogs with PH, despite parasite clearance, may be due to this persistence [5,9]. On the other hand, 12.9% of dogs without PH were observed to have increased VHS on day 0, which remained constant throughout this study. These dogs did not have any other associated pathology, as this was an exclusion criterion, and the animals were examined prior to inclusion in this study. Therefore, this could be due to the use of a generic reference value established without taking into account breed-specific indices, and some dog breeds have variations in VHS reference values [35,36].

Similarly, a significant increase in CrPA/R4 and CdPA/R9 ratios was observed throughout this study, indicating a slight worsening of arterial damage. This is consistent with previous reports by other authors, indicating persistence of endarteritis in dogs after parasite clearance [8,17]. In this research, even one of the dogs went from having normal pulmonary arterial pressure to being hypertensive at the end of the treatment, which had also been observed in a previous study [8]. Furthermore, as mentioned before, a study observed a possible persistence of inflammation at the vascular level by serological detection of acute phase proteins and endothelin-1 in dogs with PH, 7 months after the end of adulticidal treatment [18]. In this context, the results of the present study are in agreement with previous reports, showing that CrPA/R4 and CdPA/R9 ratios could be used to determine the persistence of PH after the end of adulticide treatment.

Given the accessibility of radiological techniques to veterinary clinicians, the low technical requirements, and the low cost, the results of this study are of great interest as an aid in the post-treatment evaluation of dogs with heartworm. Given the high incidence of PH in dogs with *D. immitis* and the persistence of endarteritis and PH in chronically infected dogs, the objective determination of VHS, CrPA/R4, and CdPA/R9 ratios may be useful for long-term monitoring when other imaging tools are not available or in support of other imaging tools. This could have a beneficial impact on the animal, as it would allow for close monitoring, leading to better quality and life expectancy.

5. Conclusions

In conclusion, knowledge of the response and possible changes in the pulmonary vasculature after adulticide treatment by radiological assessment of VHS, CrPA/R4, and CdPA/R9 ratios could be useful in the detection and monitoring of PH in dogs during adulticide treatment and for close monitoring thereafter.

Author Contributions: J.A.M.-A. and E.C. designed this study. S.F.-C., Y.F.-C. and E.C. wrote the manuscript. S.F.-C., Y.F.-C., J.I.M. and N.C.-R. performed the fieldwork, collected the data, and performed the experiments. All authors participated in the discussion of the results, corrected, read, and approved the final manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: The presented study was supported by own funds from the Internal Medicine Service FULP/ULPGC (SD-240/030/0026). N.C.-R. was supported by the "Grants for the predoctoral training program for researchers" program of the Government of the Canary Islands (TESIS 2021010010).

Institutional Review Board Statement: Ethical review and approval were not required for the animal in this study. All radiographs and echocardiographic measures were routinely collected for prescribed diagnostic purposes or official monitoring studies and subsequently made available to this study. All of the dog owners were informed about the present study and consented to participate. This study was carried out in accordance with the current Spanish and European legislation on animal protection (Spanish Royal Decree 53/2013 and 2010/63/UE Directive).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: All data generated or analyzed during this study are included in this article. The datasets used and/or analyzed during the present study are available from the corresponding author upon reasonable request.

Acknowledgments: The authors would like to thank Uranovet for kindly providing the Urano test Dirofilaria®. Also, they would like to thank the Hospital Clínico Veterinario of the Universidad de Las Palmas de Gran Canaria (ULPGC) for their support.

Conflicts of Interest: The authors declare no conflicts of interest.

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Radiological Evaluation of Vascular Structures in Cats Infected with Immature Worms of *Dirofilaria immitis*

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Simple Summary: This study aimed to identify thoracic radiographic abnormalities in cats infected with immature worms of *Dirofilaria immitis*. A total of 123 cats from a hyperendemic area were included and divided into healthy cats (n = 50), asymptomatic cats who were seropositive to *D. immitis* antibodies (n = 30), and seropositive cats with clinical signs (n = 43). Different radiographic measurements were assessed including the VHS and CrPA/R4, CdPA/R9, CVC/Ao, and CVC/R4 ratios. The results showed that significant differences were observed between healthy and infected cats for all except the VHS, demonstrating enlarged vasculature in cats with *D. immitis*. Moreover, 62.8% of the cats with clinical signs showed a marked bronchointerstitial pattern, while asymptomatic cats mainly (33.3%) had a mild bronchointerstitial pattern. This study highlights the importance of thoracic radiography in diagnosing and monitoring heartworm disease in cats.

Abstract: This study aimed to assess thoracic radiographic abnormalities in cats infected with immature stages of *Dirofilaria immitis* to evaluate the utility of this diagnostic technique during early infection. A total of 123 cats from a hyperendemic area were classified into three groups: asymptomatic cats seronegative to anti-*D.-immitis* antibodies (Group A), seropositive asymptomatic cats (Group B), and seropositive cats with clinical signs that were at high risk of heartworm-associated respiratory disease (HARD) (Group C). Radiographic measurements and lung parenchymal abnormalities were analyzed and compared across the groups. Significant differences in several parameters, including CrPA/R4, and CdPA/R9 ratios, were observed between healthy and seropositive cats, suggesting early arterial damage even in the absence of adult worms. Other parameters that showed differences between healthy and infected cats were CVC/Ao and CVC/R4 ratios, but not the VHS. Group C exhibited a marked bronchointerstitial pattern, indicating severe parenchymal alterations associated with clinical signs. The study demonstrated that thoracic radiography can detect early vascular and parenchymal changes in feline *D. immitis* infections, providing valuable information for diagnosing HARD. However, it also highlights the limitations of radiographic techniques, as some seropositive cats displayed no significant abnormalities. The findings underscore the importance of combining radiography with clinical and serological assessments for a more accurate diagnosis of feline heartworm disease.

Keywords: feline heartworm disease; imaging diagnosis; thoracic radiography; vector-borne disease; radiographic indexes; cardiac silhouette; vascular enlargement

Citation: Falcón-Cordón, S.; Falcón-Cordón, Y.; García-Rodríguez, S.N.; Costa-Rodríguez, N.; Vera-Rodríguez, D.J.; Montoya-Alonso, J.A.; Carretón, E. Radiological Evaluation of Vascular Structures in Cats Infected with Immature Worms of *Dirofilaria immitis*. *Animals* **2024**, *14*, 2943. https://doi.org/10.3390/ani14202943

Academic Editors: Antonio Ortega-Pacheco and Matilde Jimenez-Coello

Received: 21 August 2024
Revised: 10 October 2024
Accepted: 11 October 2024
Published: 11 October 2024

1. Introduction

Dirofilaria immitis is a nematode parasite that causes heartworm disease. It has a cosmopolitan distribution and is considered endemic in the Canary Islands [1,2]. While

cats can become infected, they are more resilient to infections with adult *D. immitis* worms compared to dogs [3]. The pathophysiology of feline heartworm is basically differentiated into two stages; the first stage is associated with the arrival of immature heartworms in the pulmonary vasculature, and the second stage is related to the presence and death of adult worms [4–6].

The first stage happens approximately 3–4 months post-infection, with the arrival of immature worms in the pulmonary arteries and arterioles and subsequent death of most of them, mainly due to the action of the intravascular alveolar macrophages. This reaction causes clinical signs due to an acute vascular and parenchymal inflammatory response [6,7]. These signs are mostly respiratory in nature and this symptomatic phase is referred to as heartworm-associated respiratory disease (HARD) [8,9]. Those larvae that manage to develop and reach adulthood cause the second stage of the disease. In general, cats have a low parasite burden and the longevity of the worms is relatively short [10].

Given the complicated diagnosis of feline heartworm, a combination of serological and imaging techniques is usually recommended to detect adult parasites. Feline heartworm disease is a dynamic disease, and all the diagnostic tests carried out should be performed and studied altogether to determine whether the animal is indeed infected by adult parasites or whether there is a high index of suspicion of infection [11–13].

Among the diagnostic techniques available, thoracic radiography is widely used for evaluating the pulmonary parenchyma and vascular structures in feline cardiopulmonary diseases [14]. In *D. immitis* infections, an enlargement of the main and peripheral pulmonary arteries has been described, characterized by the loss of the conical shape, tortuosity, and truncation of the caudal lobar branches. Additionally, parenchymal alterations are commonly observed, with diffuse or focal bronchointerstitial patterns detectable on radiographs [10,15–17]. However, these lesions are not patognomonic and are similar to the lesions found in other diseases, such as infections by *Toxocara cati* or *Aerostrongylus* spp., asthma, or allergic bronchitis [10,18–20]. In most cases, these alterations are accompanied by clinical signs such as coughing or dyspnea, among others [5,10,16]. In any case, a radiological study, used in combination with the clinical history, has proven essential for establishing a correct diagnosis [21,22].

Regarding studies related to HARD induced by immature *D. immitis* in cats, it has been reported that histopathological lesions were mainly focused on the main pulmonary artery and arterioles; this caused bronchointerstitial changes, such as bronchiolar lesions and partial obstruction of some primary bronchi, hyperplasia and hypertrophy of the muscular layer and medial hypertrophy of the small pulmonary arterioles, and interstitial lung disease [6,9,10,23]. Moreover, the studies reported that these alterations may result in pulmonary endarteritis, indicating that even transient infection can cause long-term lesions in cats [10,23] that may be detectable with thoracic radiography, which is the aim of the present study. However, other studies reported that the minor distinctions observed between infected and healthy cats indicated that clinical use of thoracic radiology was very limited or, in some cases, thoracic radiographs provided no evidence of infection in cats with immature infections [10,17]. Therefore, the aim of this study was to identify thoracic radiographic abnormalities in cats infected with immature stages of *D. immitis* to determine the utility of this diagnostic technique at this stage of infection.

2. Materials and Methods

2.1. Study Animals

A total of 123 rescued and client-owned cats brought to the Veterinary Teaching Hospital of the University of Las Palmas de Gran Canaria were included in the study. These cats lived in a hyperendemic area for *D. immitis* [1,2]. They were cats that participated in a feline heartworm screening campaign for cats over 7 months of age who never received heartworm chemoprophylaxis. Clinical history and data were recorded for each animal, including age, sex, and breed. All owners provided their consent for participation in

this study. The study was conducted in accordance with current European legislation on anima protection.

The cats were further examined for the presence or absence of clinical signs related to feline heartworm, such as coughing, dyspnea, tachypnea, increased respiratory effort, vomiting, systolic heart murmur, anorexia and weight loss, ascites, syncope, or neurological signs.

In addition, each feline patient underwent a thorough medical history and physical examination to eliminate the possibility of other conditions that could impact the results; cats with concomitant diseases were not included in the study. Additionally, testing for feline immunodeficiency virus (FIV) and feline leukemia virus (FeLV) was also carried out and any cat that tested positive was excluded from the study.

Out of the 123 cats surveyed, 42.35% (52/123) were male while 57.65% (68/123) were female. There were 170 European Shorthair cats, 2 Sphynx cats, 1 Maine Coon cat, and 1 Turkish Angora cat included in the breed categorization.

2.2. Serology

Blood samples were taken from either the cephalic or jugular vein and spun in dry tubes to check their serological status. The serum was stored at a temperature of −20 °C until the tests were conducted. The presence of feline *D. immitis* infection was determined through serological methods to detect specific antibodies against *D. immitis* using an indirect enzyme-linked immunosorbent assay (ELISA) (in-house ELISA, Urano Vet®, Barcelona, Spain). In short, each well of the ELISA plate was covered with recombinant *D. immitis* antigens (Di33 protein, 0.5 ug/mL). The sample diluent was mixed with the serum in a 1:100 dilution ratio. Following an initial wash to eliminate unbound molecules, the TMB substrate, labeled with horseradish peroxidase, was introduced, targeting feline IgG specifically. The readings of absorbance (or optical density) were taken at 450 nm within a 5-min time frame following the introduction of the stop solution (sulfuric acid). As per the kit manufacturer's guidelines, seronegativity was defined at a cut-off of < 1, while seropositivity was defined at a cut-off of 1 or higher. Furthermore, all samples were tested for circulating *D. immitis* antigens using a commercial immunochromatographic test kit (Uranotest Dirofilaria ©, UranoVet SL, Barcelona, Spain) according to the manufacturer's instructions.

2.3. Imaging Diagnosis

Echocardiograms were conducted on every cat to confirm the presence or absence of adult worms, as well as to exclude any other concurrent diseases. The cats were positioned on their right side with the transducer positioned in the third intercostal space to check for worms in the pulmonary arteries and right-sided heart chambers. Cats remained conscious and were continuously monitored with electrocardiography throughout the entire test.

Thoracic radiographs from all cats were taken using the same radiographic equipment (Bennett HFQ-600P, Greensboro, NC, USA) during inspiration and without sedation to minimize changes in heart size [24]. Views were obtained in both right laterolateral and dorsoventral projections, and radiographic measurements were taken using adjustable calipers by an unbiased operator (SFC), who has 10 years of clinical experience in cardiorespiratory diseases in small animals and was blinded to the clinical status of the study cats (Figure 1).

In lateral recumbent radiographs, the Vertebral Heart Score (VHS) measurement was obtained from the sum of the short-axis and the long-axis measurements as previously described [25]. The cardiac long axis was obtained from the cardiac apex to the base of the heart where it meets the trachea just cranial to the carina, expressed as the number of vertebral lengths in the lateral radiograph, measured caudally from the cranial border of T4. The short axis of the heart was measured perpendicular to the long-axis measurement at the point of maximum heart width, expressed as the number of vertebral lengths in the lateral radiograph, measured caudally from the cranial border of T4 [26].

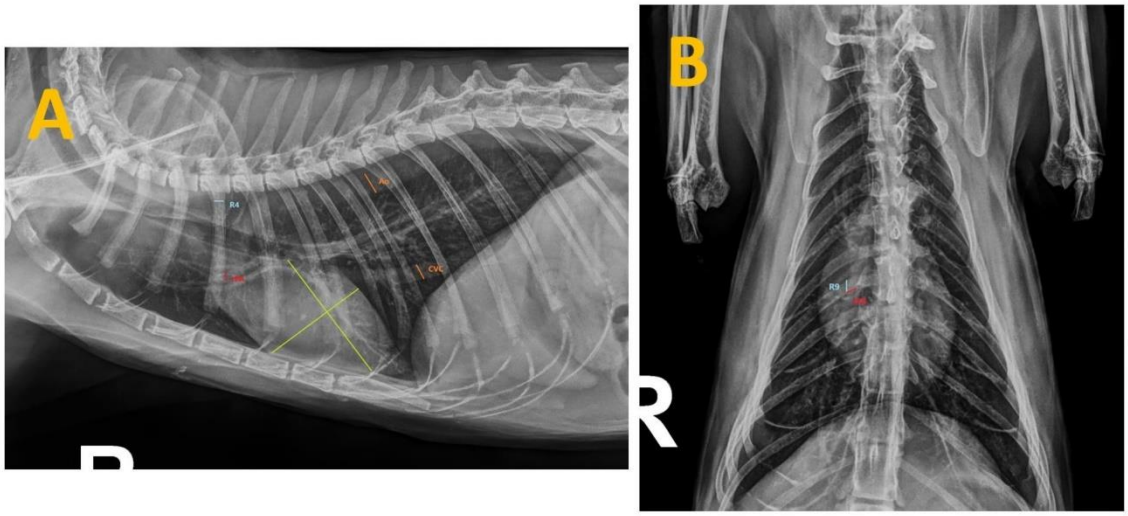

Figure 1. Thoracic radiographs of a cat seropositive for anti-Dirofilaria-immitis antibodies and presenting with clinical signs. The measurements taken during this study are shown as follows: (**A**) Right laterolateral projection illustrating the measurements of the caudal vena cava (CVC) and aorta (Ao) (in orange), the fourth rib (R4) (in blue), the right cranial pulmonary artery (CrPA) (in red) and the vertebral heart score (in green). (**B**) Dorsoventral projection displaying the measurement of the right caudal pulmonary artery (CdPA) (in red) in relation to the ninth rib (R9) (in blue).

In the laterolateral projection, the diameter of the right fourth rib (R4) just below the spine and the greatest diameter of the caudal vena cava (CVC) were also measured as described in previous studies, which included dogs with heartworm, in order to determine the mean caudal vena cava size, expressed as a ratio of the diameter of R4 [27–29]. The measurement of the diameter of the descending aorta (Ao) at the same intercostal space as the CVC was carried out as well, following previous guidelines in dogs [29]. Next, the CVC/Ao and CVC/R4 ratios were established.

Other radiological measurements were taken, including the diameter of the right cranial pulmonary artery (CrPA) passing through R4 in the laterolateral projection and the diameter of R4 at a point just distal to the spine. Moreover, in the dorsoventral projections, the distal and left sides of the summation shadow created by the right caudal pulmonary artery (CdPA) with R9 were measured. Finally, the CrPA/R4 and CdPA/R9 ratios were calculated from these measurements [30].

In addition, quantitative evaluation of the shape and tortuosity of the lung vasculature in both radiographic projections took place. Finally, the parenchyma of all radiographs was examined to determine the presence or absence of radiological abnormalities and their nature, classified as bronchial pattern, vascular pattern, interstitial pattern, alveolar pattern, or mixed patterns in all groups.

2.4. Statistical Analyses

The data were analyzed using SPSS Base 29.0 software for Windows. A Shapiro–Wilk test was performed to verify the normal distribution of the data. Additionally, a Siegel–Tukey test was performed to verify the variability of variances between groups. The chi-squared test was used to assess the association between categorical variables. A non-parametric Mann–Whitney U test was performed to determine differences between the groups for all recruited-cat measurements. In all cases, a p-value < 0.05 was determined as significant. Continuous variables were expressed as the median $\pm$ standard deviation,

while qualitative variables were expressed as percentages. In all cases, a p-value < 0.05 was considered significant. In addition, Pearson's correlation coefficient was obtained to determine the relationship between variables. The strength of the correlations was categorized according to standard conventions: a correlation coefficient of $r \leq 0.30$ was considered weak, $0.31 \leq r \leq 0.50$ was classified as moderate, and $r > 0.50$ was regarded as strong.

3. Results

Based on the results, cats were divided into three groups: Group A (n = 50) consisted of cats with no clinical signs that were seronegative for anti-*D.-immitis* antibodies, Group B (n = 30) consisted of cats seropositive to *D. immitis* but who were asymptomatic, and Group C (n = 43) comprised seropositive animals with *D. immitis* exhibiting clinical signs included in the differential diagnosis of HARD. Antigen tests were negative in all cats included in the study. The list of clinical signs observed in the cats of Group C can be seen in Table 1.

Table 1. Summary of clinical signs observed in the study cats from Group C. Legend: n = number of cats showing the clinical sign described. Percentage (%) = percentage of cats in Group C showing the described clinical sign.

Clinical Sign	Number of Cats (n)	Percentage (%)
Cough	18	41.9%
Tachypnea	14	32.6%
Respiratory distress	23	53.5%
Vomiting	4	9.3%

The average age of the cats in the study was 4.60 ± 3.36 years, with Group A having an average age of 4.64 ± 3.33 years, Group B 4.27 ± 3.66 years, and Group C 4.67 ± 3.40 years. No significant statistical differences were found in age among the groups. The average weight of the cats was 3.72 ± 1.11 kg (3.51 ± 0.56 kg for cats from Group A, 3.86 ± 1.03 kg for cats from Group B, and 3.89 ± 1.57 kg cats from Group C). There were no significant differences in weight between the groups. During the echocardiographic study, adult parasites were not found in any of the cats.

No quantitative abnormalities in the shape or tortuosity of the pulmonary vasculature were found on any of the radiographs studied. The obtained radiographic measurements are shown in Table 2. A VHS value for Group A was determined as 6.43 ± 0.92 (with an upper limit of 7.3), with no significant differences between the groups in terms of the VHS value. However, 37.21% (10/43) of cats from Group C showed cardiomegaly based on established reference values [25].

For the CVC/Ao and CVC/R4 ratios, the results from Group A showed a mean value of 1.09 ± 0.12 (upper limit 1.22) and 1.62 ± 0.15 (upper limit 1.77), respectively, being significantly higher in the seropositive cats for both ratios (Table 2). Moreover, statistically significant differences were present between Groups B and C ($p = 0.003$ for CVC/Ao and $p = 0.021$ for CVC/R4).

The mean value for the CrPA/R4 ratio for Group A was 0.76 ± 0.05 (upper limit 0.81). Statistically significant differences were observed between healthy cats and the rest of the studied groups ($p < 0.001$); however, no significant differences were observed between Groups B and C for this parameter (Table 2).

For ventrodorsal projection, the obtained ratio for CdPA/R9 was 0.79 ± 0.05 (upper limit 0.84). Statistically significant differences were observed between Group A and the rest of the studied groups ($p < 0.001$). Also, a statistically significant difference was presented between Group B and Group C ($p = 0.017$) (Table 2).

To determine if there is a correlation between the parameters with age and weight, the Pearson correlation model was used. Initially, the results showed a low positive or negative correlation between radiographic measurements and age or weight, and only

a moderate positive correlation was observed between CdPA/R9 and weight. Finally, a moderate positive correlation was determined between the CVC and VHS (Table 3).

Table 2. Results expressed by radiographic measurements and groups. Legend: VHS (Vertebral Heart Size); CrPA/R4 (right cranial pulmonary artery passing through the fourth rib in the laterolateral projection ratio); CVC/Ao (ratio of the caudal vena cava and diameter of the descending aorta in the laterolateral projection); CVC/R4 (caudal vena cava expressed as a ratio of the diameter of the fourth rib in the laterolateral projection); CdPA/R9 (right caudal pulmonary artery to the ninth rib in the dorsoventral projection ratio). Group A: seronegative asymptomatic cats; Group B: asymptomatic cats seropositive to anti-*Dirofilaria-immitis* antibodies; Group C: cats with clinical signs that were seropositive to *D. immitis*. Results are expressed as mean ± standard deviation.

Measure	Groups	Results	*p*-Value	R Effect	Interpretation
VHS	**Group A**	6.43 ± 0.92	0.27^{ns}	0.05472692	No statistically significant differences between seronegative and seropositive cats
	Group B	5.98 ± 1.04	0.41655099^{ns}	0.02500844	No statistically significant differences between Groups B and C
	Group C	6.61 ± 1.62			
CrPA/R4	**Group A**	0.76 ± 0.05	3.0545×10^{-21} **	0.74419009	Statistically significant difference between seronegative and seropositive cats
	Group B	1.12 ± 0.22	0.41783262^{ns}	0.02427917	No statistically significant differences between Groups B and C
	Group C	1.17 ± 0.19			
CVC/Ao	**Group A**	1.09 ± 0.12	5.02886×10^{-07} **	0.44459144	Statistically significant difference between seronegative and seropositive cats
	Group B	1.16 ± 0.14	0.00349851 **	0.31565838	Statistically significant difference between Groups B and C
	Group C	1.35 ± 0.25			
CVC/R4	**Group A**	1.62 ± 0.15	1.2276×10^{-21} **	0.74896233	Statistically significant difference between seronegative and seropositive cats
	Group B	2.41 ± 0.61	0.021834542 *	0.23490465	Statistically significant difference between Groups B and C
	Group C	2.62 ± 0.47			
CdPA/R9	**Group A**	0.79 ± 0.05	8.7566×10^{-22} **	0.75130749	Statistically significant difference between seronegative and seropositive cats
	Group B	1.23 ± 0.31	0.017873607 *	0.24919937	Statistically significant difference between Groups B and C
	Group C	1.36 ± 0.34			

**, Correlation is significant at 0.5% ($p < 0.005$); *, correlation is significant at 5% level ($p < 0.05$); ns, correlation is not significant.

Table 3. Correlation coefficient for all studied parameters with weight and age values, as well as correlation between CVC and VHS.

Correlation	Coefficient	Interpretation
VHS–Weight	0.26410673 **	Low positive correlation
VHS–Age	0.2625266 *	Low positive correlation
CrPA/R4–Weight	0.24220781 *	Low positive correlation
CrPA/R4–Age	-0.02621345^{ns}	Low negative correlation
CVV/Ao–Weight	0.27288863 **	Low positive correlation
CVV/Ao–Age	-0.06015906^{ns}	Low negative correlation
CVV/R4–Weight	−0.0537349	Low negative correlation
CVV/R4–Age	-0.1179146^{ns}	Low negative correlation
CdPA/R9–Weight	0.41493029 **	Moderate positive correlation
CdPA/R9–Age	0.0134005^{ns}	Low positive correlation
VCC–VHS	0.4903414 **	Moderate positive correlation

**, Correlation is significant at 0.5% ($p < 0.005$); *, correlation is significant at 1% level ($p < 0.01$); ns, correlation is not significant.

Regarding the obtained results for pulmonary patterns, Group A cats did not show any lung abnormalities. The results pertaining to cats in Groups B and C showed various lung parenchymal abnormalities including bronchial, mild and marked bronchointerstitial, and alveolar patterns (Table 4). The findings indicated that the predominant pulmonary pattern in Group B was a mild bronchointerstitial pattern (33.3%; 10/30), while in Group C, a marked bronchointerstitial pattern was observed in 62.8% (27/43) of cats. On the other hand, 30% (9/30) of cats in Group B showed no radiological abnormalities at the level of the lung parenchyma. A chi-squared test was performed to confirm the correlation between the presence/absence of clinical signs and the presence/absence of lung parenchymal abnormalities in cats from Groups B and C, irrespective of the type of radiological lung abnormality and its severity. A strong correlation was found between the presence of clinical signs and the presence of lung parenchymal abnormalities, with a statistically significant difference ($p < 0.001$) (Table 4).

Table 4. Lung parenchymal abnormalities observed in the studied cats. Group B: asymptomatic cats seropositive to anti-*Dirofilaria-immitis* antibodies; Group C: cats with clinical signs that were seropositive to *D. immitis*. Results are expressed as percentage (%) as well as number of cats exhibiting each lung pattern by group.

Lung Parenchymal Abnormalities	Group B (n = 30)	Group C (n = 43)	Groups B + C (n = 73)
Bronchial pattern	20% (6/30)	4.6% (2/43)	10.9% (8/73)
Bronchointerstitial pattern (mild)	33.3% (10/33)	13.9% (6/43)	21.9% (16/73)
Bronchointerstitial pattern (marked)	16.7% (5/30)	62.8% (27/43)	43.8% (32/73)
Alveolar + interstitial pattern	0% (0/30)	18.6% (8/43)	10.9% (8/73)
Total	70% (21/30)	100% (43/43)	87.6% (64/73)

4. Discussion

The diagnosis of heartworm infection in cats is far more complex than in dogs due to the specific characteristics of the feline host, such as a low parasite load [3,10]. In the case of juvenile or pre-adult worm infections, diagnosis is virtually impossible and therefore very complicated. Thus, the objective identification of compatible radiographic changes may be useful as an indicator of a high suspicion of HARD in infected cats. In infections with adult parasites, thoracic radiography is a valuable diagnostic and monitoring tool for the diagnosis of feline heartworm disease [26,31]. However, it has never been objectively assessed in cats with high suspicion of HARD. Radiographic abnormalities may be less consistent in feline heartworm disease than in canine heartworm disease, and the absence of such abnormalities does not exclude the diagnosis of heartworm disease in cats [3,12,32]. Therefore, this study was undertaken to evaluate whether specific and objective radiographic features of the heart and pulmonary vasculature could aid in the diagnosis of HARD.

The VHS values for healthy cats were similar to those previously reported by other authors (6.7–8.1, mean 7.5) [25,33]. The results indicated no significant differences in the VHS between the different groups, in contrast to what was reported by Litster et al. [26], who found that the mean VHS for the heartworm-infected cats was significantly greater than the reference value, and Venco et al. [17], who observed a tendency for the heart silhouette to increase in size during infection and at the onset of clinical signs. These differences may be attributed to the fact that the present study focused on cats with early infections, whereas the cited studies [17,26] were performed in cats with adult parasites. Nevertheless, it should be noted that 10 cats in Group C showed cardiomegaly, suggesting a trend towards an increased cardiac silhouette in cats with clinical signs, similar to that reported by Venco et al. [17]. However, the small variations in the analyzed parameters compared to healthy cats indicate the minimal usefulness of these measures as clinical diagnostic tools [17,26]. Indeed, the heart is rarely affected in feline heartworm disease [6].

A moderate (almost strong) positive correlation between the VHS and the diameter of the CVC was identified. Quite similar results (r = 0.59) were previously obtained in cats infected with *D. immitis* [26]; these authors reported that this finding, along with the increased mean VHS, may be linked to an elevation in filling pressures during the infection. Additionally, the CVC/Ao ratios were increased in infected cats compared to the healthy group; however, while the CVC/Ao ratio may provide some insights into right-sided heart conditions, pulmonary hypertension, and right ventricular hypertrophy, these are not typically observed in cats infected with *D. immitis* [6,10,15]. Similar findings were reported by Litster et al. [26], who found that the maximum width of the CVC in heartworm-infected dogs and cats was significantly greater than that obtained in the reference group, suggesting elevated right-sided heart filling pressures in both species. The authors argued that increased cardiac size and elevated filling pressures correlated and progressed together with heartworm disease. However, while the present study has shown an increased CVC/Ao ratio in infected cats, other potential factors influencing this measurement should be considered, and it should not be solely attributed to right-sided heart disease. Moreover, the echocardiographic exam carried out in the studied cats showed no evidence of right-sided heart disease. Further research is necessary to clarify the relationship between the CVC/Ao ratio and right-sided heart disease specifically in cats, given the unique aspects of feline heartworm infection.

Regarding the results obtained for the CVC/R4 ratio, similar results were observed, with the highest mean values seen in Group C. These findings are similar to those of other studies that examined the CVC/R4 ratio in dogs with heartworm with varying degrees of cardiac enlargement and found that this ratio increased with the severity of right ventricular enlargement [27,28]. The authors stated that, assuming that the degree of right ventricular enlargement was directly related to the severity and duration of heartworm infection, the relationship between CVC and right ventricular enlargement may reflect an increase in central venous pressure due to impending *cor pulmonale* [27]. This finding would be similar to that reported in cats, as discussed earlier [26], where it was suggested that increased cardiac size and elevated filling pressures occurred and were proportional to each other as the cardiac effects of heartworm disease progressed. Although the CCV/R4 ratio is not yet widely used in feline cardiology, these canine studies, along with the aforementioned study conducted in cats with heartworm, provide a reasonable basis for hypothesizing that an increased CCV/R4 ratio may indicate cardiac stress in cats with HARD, just as other authors have seen an increased VHS in cats with *D. immitis* showing clinical signs [17]. However, as mentioned earlier, further research is needed to determine the diagnostic utility of this ratio in feline heartworm disease.

The CrPA/R4 ratios obtained for healthy cats were very similar to those previously reported, which were 0.7 ± 0.13 [30]. The results showed that this ratio was significantly increased in cats with heartworm and, regardless of the presence or absence of clinical signs, these values were elevated in a large proportion of the animals infected with *D. immitis*. This differs from that reported by other authors, who subjectively reported that cranial lobar vessels were not enlarged in cats with heartworm [32]. However, previous studies found that cats infected with immature parasites had a significant increase in wall thickness, with occlusive medial hypertrophy present in 50% of cats infected by immature worms [34]; moreover, these authors argued that it was possible that medial hypertrophy of the small pulmonary arteries in exposed cats represented a pathologic response to transient heartworm infection. The first detectable pulmonary lesions of *D. immitis* infection include arteritis, pneumonitis, and hypertrophy of smooth muscle cells in the tunica media of small pulmonary arteries, which are likely attributable to pulmonary embolization of fifth-stage larvae before the establishment of infection with adult heartworms [35,36]. Therefore, it would be expected that certain arterial abnormalities would be found in the thoracic radiographs of these cats.

Similarly, the results showed values for CdPA/R9 ratios in healthy cats within the reference ranges usually considered (<1) [37]. Other authors have established significantly

higher reference values for healthy cats (1.37 ± 0.28 for CdPA/R9) [32]. The reason for these differences beyond interobserver variability is unknown. Several authors agreed that a CdPA/R9 ratio greater than 1.6 has previously been reported in association with feline heartworm disease [26,32]. The results of the present study do not show such a pronounced thickening, likely because the cats examined were in the early stages of infections with immature parasites, while other studies involved chronic infections with adult parasites and, therefore, more advanced arterial damage [9]. However, the presence of significant differences between the seropositive cats and the Control Group is indicative of vascular lesions; moreover, these ratios were higher in cats with clinical signs. As noted above, in HARD, occlusive medial hypertrophy of the small pulmonary arterioles occurs, as well as changes in the pulmonary arteries [6]. Death of *D. immitis* in the pre-cardiac stages can also lead to smooth muscle hypertrophy of pulmonary arterioles.

In cats with HARD, changes are observed in the bronchi, bronchioles, and alveoli [5,6,23,34]. In this study, the lung patterns observed were more severe in feline patients with clinical signs, as lung parenchymal abnormalities were also observed in asymptomatic patients. Cats with clinical signs showed predominantly marked bronchointerstitial patterns, while asymptomatic cats showed milder forms. This is consistent with the pathophysiology of the feline heartworm and the pathogenesis of HARD, which is caused by the death of immature worms upon reaching the lungs. Obviously, this leads to clinical signs and radiological changes. Conversely, other studies have reported no correlation between radiographic lesions, clinical signs, or antibody levels [38]; however, this was a study based on a small number of cats (n = 10), all infected with adult worms. On the other hand, it has been described that infected cats may present with apparently normal thoracic radiographs [3,10], as observed in this study. The cardiopulmonary response to heartworm infection is dynamic and radiographs may not show changes if they are produced very early or very late in the course of the disease [11]. When present, feline heartworm should be considered in cats whose clinical and epidemiological characteristics are consistent with the infection.

A cat can remain seropositive for anti-*D.-immitis* antibodies for up to a year after clearance of the infection [23], so the seropositivity of the study cats does not necessarily indicate active infections, especially in asymptomatic cats, which is a limitation of this study. Moreover, the radiographic lesions of feline heartworm infection are dynamic over time, as demonstrated in experimental cat models where the timing of infection was known with accuracy [6,10,38]; as these cats were naturally infected, the exact time of infection is unknown, which may have affected the results. Nonetheless, these were infected cats that had been exposed to larval forms of the parasite in a hyperendemic region. In a study in which cats were experimentally inoculated with 100 L3 and subsequently treated with macrocyclic lactones as early as 70 days after infection, they showed radiographic and histopathological changes consistent with HARD at necropsy [5,23]. In addition, a large study with client-owned cats showed that 28% of heartworm-infected cats were asymptomatic [39].

5. Conclusions

The radiographic changes observed in the cats of this study indicated the presence of vascular and parenchymal abnormalities in those likely infected by immature *D. immitis* parasites. This was particularly evident in cats exhibiting clinical signs consistent with HARD, suggesting early vascular damage caused by this parasite. Given the challenges in diagnosing infections by immature *D. immitis* worms in cats, the examination of these radiographic measurements could serve as a valuable diagnostic tool for veterinary clinicians when HARD is strongly suspected. The results of the present study support the diagnostic suspicion of HARD in cats with compatible clinical signs and radiographic findings; however, heartworm infection should not be entirely ruled out in cats showing normal thoracic radiographs.

Author Contributions: J.A.M.-A. and E.C. designed the study. S.F.-C., Y.F.-C. and E.C. wrote the manuscript. S.F.-C., Y.F.-C., S.N.G.-R., D.J.V.-R. and N.C.-R. performed the fieldwork, collected the data and performed the experiments. All authors participated in the discussion of the results and correction of the final manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: The presented study was supported by funds from the Internal Medicine Service FULP/ULPGC (SD-240/030/0026). NCR was supported by the "Grants for the predoctoral training program for researchers" program of the Government of the Canary Islands (TESIS2021010010), financed by the Consejería de Economía, Industria, Comercio y Conocimiento, and the Fondo Social Europeo. SNRG was supported by the "Grants for the financing of predoctoral contracts" program of the Universidad de Las Palmas de Gran Canaria (PIFULPGC-2020-2-CCSALUD-2).

Institutional Review Board Statement: Ethical review and approval were not required for the animals in this study. All radiographs and echocardiographic measures were routinely collected for prescribed diagnostic purposes or official monitoring studies and subsequently made available for this study. All of the cat owners were informed about the present study and consented to participate. The study was carried out in accordance with the current Spanish and European legislation on animal protection (Spanish Royal Decree 53/2013 and 2010/63/UE Directive).

Informed Consent Statement: Not applicable.

Data Availability Statement: All data generated or analyzed during this study are included in this article. The datasets used and/or analyzed during the present study are available from the corresponding author upon reasonable request.

Acknowledgments: The authors would like to thank Uranovet for kindly providing the Urano test Dirofilaria® and the in-house ELISA Urano Vet®. Also, they would like to thank the Hospital Clínico Veterinario of the Universidad de Las Palmas de Gran Canaria (ULPGC) for their support.

Conflicts of Interest: The authors declare no conflicts of interest.

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CONCLUSIONS

1. An enlargement of the cardiac silhouette did not seem to be helpful in determining the presence or absence of pulmonary hypertension in dogs infected by *Dirofilaria immitis*. Nonetheless, the results for the CrPA/R4 and CdPA/R9 ratios indicated potential cut-off values with reasonable levels of sensitivity and specificity when compared to echocardiographic determination of pulmonary hypertension. These findings may recommend considering these ratios in the initial assessment of thoracic radiographs in dogs as a screening method before deciding on further diagnostic tests for the assessment of pulmonary hypertension.

2. The Vertebral Heart Score (VHS), CrPA/R4, and CdPA/R9 ratios remained significantly increased in dogs with pulmonary hypertension throughout adulticidal treatment against *Dirofilaria immitis*, at least up to 6 months after the end of treatment. These results are in line with studies reporting that pulmonary hypertension may not be reversible in dogs with heartworm and continuous surveillance is thus essential for the effective post-treatment management of pulmonary hypertension in these animals. In this sense, knowledge of the response and possible changes in the pulmonary vasculature after adulticide treatment by radiological assessment of VHS, CrPA/R4, and CdPA/R9 ratios could be useful in the detection and monitoring of pulmonary hypertension in dogs during adulticide treatment and for close monitoring thereafter.

3. The radiographic changes observed in the CrPA/R4, CdPA/R9, CVC/Ao and CVC/R4 ratios of the cats of this study, as well as the abnormal patterns observed at the level of the lung parenchyma, indicated the presence of vascular and parenchymal abnormalities in those likely infected by immature *Dirofilaria immitis* parasites. This was particularly evident in cats exhibiting clinical signs consistent with heartworm-associated respiratory disease (HARD), suggesting early vascular damage caused by this parasite. Given the challenges in diagnosing infections by immature *D. immitis* worms in cats, the examination of these radiographic measurements could serve as a valuable diagnostic tool for veterinary clinicians when HARD is strongly suspected. However, heartworm infection should not be entirely ruled out in cats showing normal thoracic radiographs.

SIMPLE SUMMARY

The nematode *Dirofilaria immitis* primarily affects domestic and wild carnivores. This parasite resides in the pulmonary arteries and the right ventricle of the heart, and it is mainly transmitted by mosquitoes of the genera *Culex*, *Aedes*, and *Anopheles*. The inflammation of the vascular endothelium caused by the mechanical action of adult parasites chronically leads to proliferative endarteritis, pulmonary hypertension (PH), and congestive heart failure. In dogs, clinical signs usually include chronic cough, exercise intolerance, and weight loss, and the infection can be fatal if not treated in time. In cats, asymptomatic cases are frequent; however, both aberrant migration of the parasites and the presence of adult worms can cause sudden death. Diagnosis is mainly based on the detection of circulating *D. immitis* antigens. In felines, specific antibodies against the parasite can also be detected. Furthermore, diagnostic imaging techniques are valuable complementary diagnostic tools, both for diagnosis and determining the clinical status of the infected animal. Among the techniques used, echocardiography and thoracic radiography stand out.

This thesis evaluates whether changes in certain radiographic indices (VHS, CrPA/R4, and CdPA/R9) can serve as indicators of PH in dogs with heartworm, both for preliminary diagnosis and for follow-up after adulticide treatment. The results determined that the CrPA/R4 and CdPA/R9 ratios proved to be effective predictors of the presence of PH, and hypertensive dogs exhibited significantly elevated values for these indices. Moreover, PH persisted in the affected dogs throughout the treatment and for at least 6 months after its completion, indicating that PH persists even after the elimination of adult parasites. Additionally, the VHS, CrPA/R4, and CdPA/R9 indices also remained elevated in hypertensive dogs throughout the study.

On the other hand, the study in cats investigated the effects of infections by immature worms and the difficulty in diagnosing the disease due to the low parasitic load. Vascular and pulmonary alterations were identified in infected cats, mainly through the CrPA/R4, CdPA/R9, and CVC/Ao ratios, as well as broncho-interstitial patterns in the lungs. Cats with immature worms showed signs of early vascular damage, even in the absence of adult worms. Cats with clinical signs showed more severe alterations, such as marked broncho-interstitial patterns on radiographs. Given that infections by immature worms are difficult to diagnose, radiographs can provide valuable clues for early diagnosis, especially when clinical signs compatible with heartworm-associated respiratory disease (HARD) are observed.

This PhD thesis highlights the importance of radiographic measurements (VHS, CrPA/R4, CdPA/R9) as useful tools for the initial detection and monitoring of PH in dogs with heartworm. Although adulticide treatment can improve some cases, PH often persists, underscoring the need for long-term monitoring. Thoracic radiographs are an accessible technique for veterinarians and can facilitate the management of this disease in clinics where advanced echocardiography is not available. In cats, although the diagnosis is more complex, radiographs can detect early alterations caused by infections with immature worms, making them essential for a preliminary diagnosis of

HARD. Long-term monitoring is crucial in both species, as the disease can persist even after treatment.

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