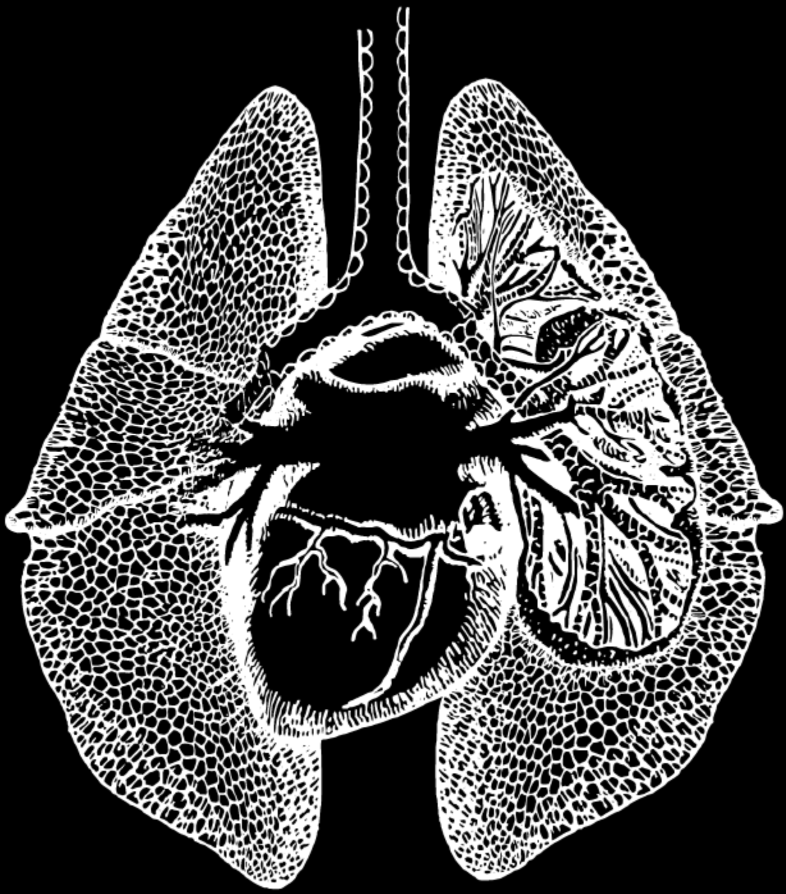

Doctorada de Investigación en Biomedicina
Tesis Doctoral con Mención Internacional
Diciembre del 2024

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

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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

CVS Group
Passionate about animal care

Barton Veterinary Hospital & Surgery
34 New Dover Road
Canterbury
Kent CT1 3BH
T. (01227) 765522
F. (01227) 786406
enquiries@barton-vets.co.uk

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 cualquier, martes o jueves durante el curso. Acabábamos de llegar de pasar consultas el grupo de becarios que en el 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 Immtis*. 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, 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 que 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 qué en nuestras Islas predomina *Dirofilaria immitis* y no *repens*. Yo no me lo podía creer, tenía que llamar a los jefes los 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 éste un poco más menguado que el anterior. Se trataba de *Dirofilaria repens*. Efectivamente amigos, pleno. Tenía en mi casa a dos filarias. Que 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 que Elena pusiese orden entre ellas a la vuelta de las vacaciones. En ese momento, me di cuenta 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 empezó 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 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.

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Tampoco puedo olvidarme de mi Raque, mi bichota, mi compañera de batallón durante estos últimos meses, nunca olvidaré tus palabras, tu apoyo, mas 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 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 mas si cabe, nos hemos visto en la duras y en las maduras y que alegría siento de decir que he contado contigo siempre así que esto ya no hay nada que lo separe, para siempre en mi corazón.

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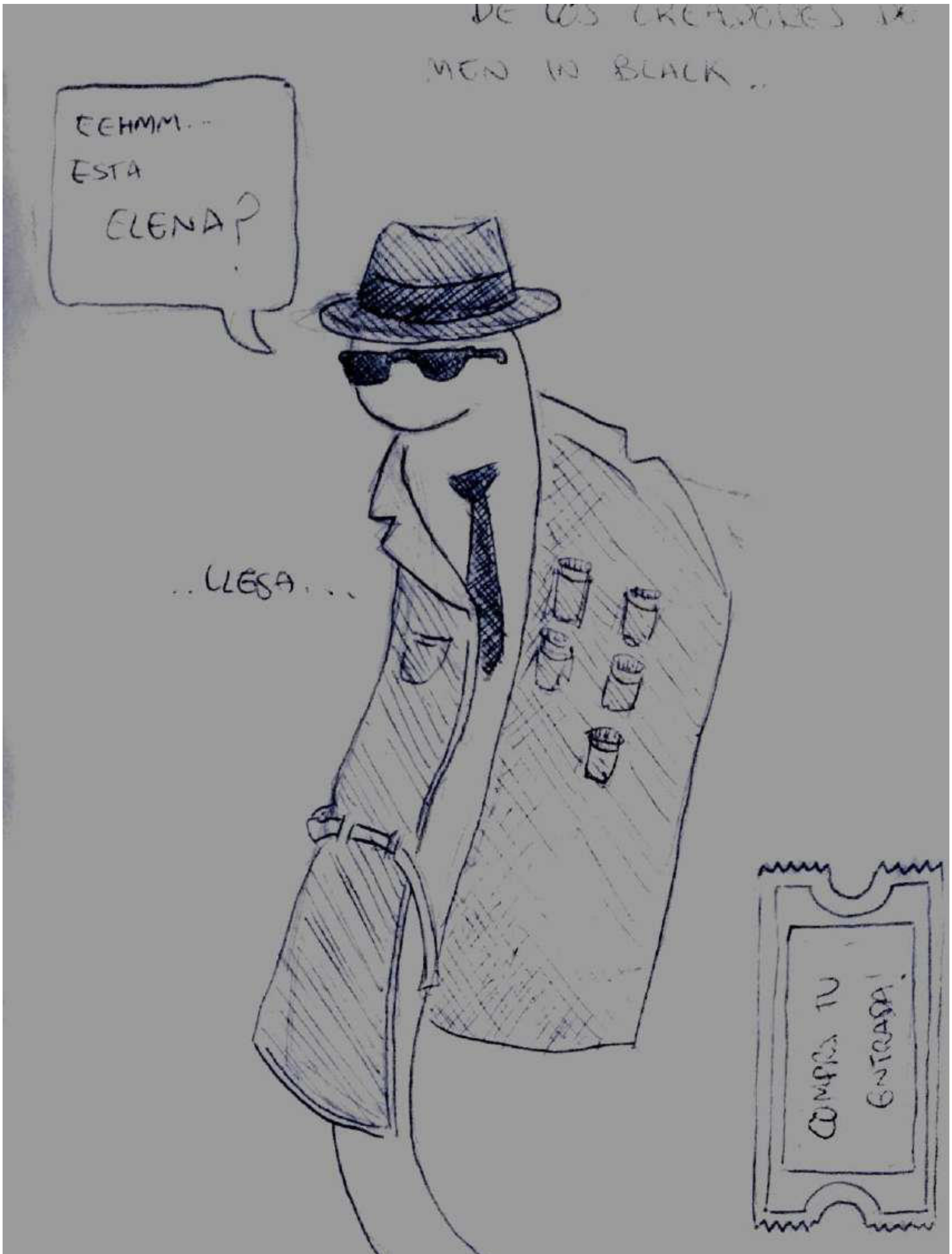
Gracias.


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

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FIRTS SECTION: PERTAINING TO THE PARASITE

INTRODUCTION

Canine heartworm disease is caused by the nematode *Dirofilaria immitis*, this parasite belongs to the genus *Dirofilaria*. It primarily affects domestic and wild carnivores. The adult forms reside in the pulmonary arteries and right ventricle of the heart. The vectors are hematophagous mosquitoes belonging to the genera *Culex*, *Aedes*, and *Anopheles* primarily. Currently, the spread of the disease is linked to the movement of infected animals from endemic areas to previously disease-free areas, the existence of wild animals acting as reservoirs (Lee et al., 2010), and the advancement of the vector due to climate change favoring the mosquito's survival and adaptation (Anvari et al., 2019a).

In most cases, the infected patients will be asymptomatic or could show mild signs of disease. Although, once clinical signs appear, it can be fatal if not treated promptly (Bowman and Atkins, 2009). Obstruction caused by adult parasites and chronic local inflammatory lesions over time lead to the appearance of symptoms such as chronic cough, exercise intolerance, weight loss, epistaxis and/or dyspnea.

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

TAXONOMY

D. immitis is a filiform nematode with a whitish color, belonging to the Class *Nematoda*, Family *Filariidae*, Genus *Dirofilaria* (Urquhart et al., 2001; Marquardt et al., 2000). Within this genus, there are different species that can parasitize dogs, *D. immitis*, *D. repens*, *Dipetalonema reconditum*, and *Dipetalonema dracunculodie*, all of them producing microfilaremia during their parasitic development. In adittion, species *D. immitis* and *D. repens* stand out for their zoonotic character and wide distribution.

Morphologically, adult worms are characterized by their cylindrical, elongated appearance. *D. immitis* exhibits marked sexual dimorphism (Levine, 1978). The female is usually larger than males, measuring up to 300 mm (Manfredi et al., 2007) whereas males could be up to 200 mm long. 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. Regarding males, they have unequal spicules and lack a gubernaculum (Mehlrom 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 by females into the bloodstream, continuing their development in the blood and other tissues of the definitive host. They can be found in the blood at any time, although, it has been reported, there is a higher number in peripheral blood at certain times of the day and season (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 *Dirofilaria immitis*, *Dirofilaria 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 migrates 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 8 months, adult females can measure between 20 to 30 cm in length. The average lifespan of the parasite is 5 to 7 years and up to 30 months for microfilariae (Bowman and Atkins, 2009). Transplacental transmission of microfilariae from mother to puppies has been described, although as immature forms, L1 larvae are not infective. The same occurs with microfilariae transmitted through blood transfusion (Mantovani and Jackson, 1966; Brinkmann et al., 1976; Todd and Howland, 1983; Menda, 1989).

On the other hand, cats are considered susceptible hosts of *D. immitis*, but not definitive hosts. Host resistance is greater in this species, and therefore the parasitic burden of adult worms is usually relatively low in naturally infected cats (2 to 4 worms). The prepatent period in this species is 8 months, with microfilaremia being infrequent and of short duration (<20% of cats with adult worms of both sexes). In turn, the parasite's median survival lifespan is shorter in this species, being a maximum of 4 years, and the parasite's length is smaller in this species.

EPIDEMIOLOGY

Climate and ecological factors directly influence the spread of vector-borne diseases in the European continent, as the development and maintenance of mosquitoes depend directly on climatic conditions (Genchi et al., 2011 a,b). The temperature and ambient humidity must be ideal for the development of mosquito larvae. These conditions ensure the parasite's life cycle (Kalluuri et al., 2007; Genchi et al., 2009), thus affecting the seasonality of *D. immitis* transmission, being higher during the summer months (Genchi et al., 2005, 2009). On the other hand, depending on the species, their hematophagous activity differs, with *Cx. Pipiens* and *Anopheles spp* being active only at night, while *An. maculipennis* and *Ae. Albopictus* are active at dawn or throughout the day. Additionally, some species show two peaks of activity: at dusk and at dawn, such as *Ae. caspius* (Mattingly, 1969; Di Sacco et al., 1992; Pollono et al., 1998).

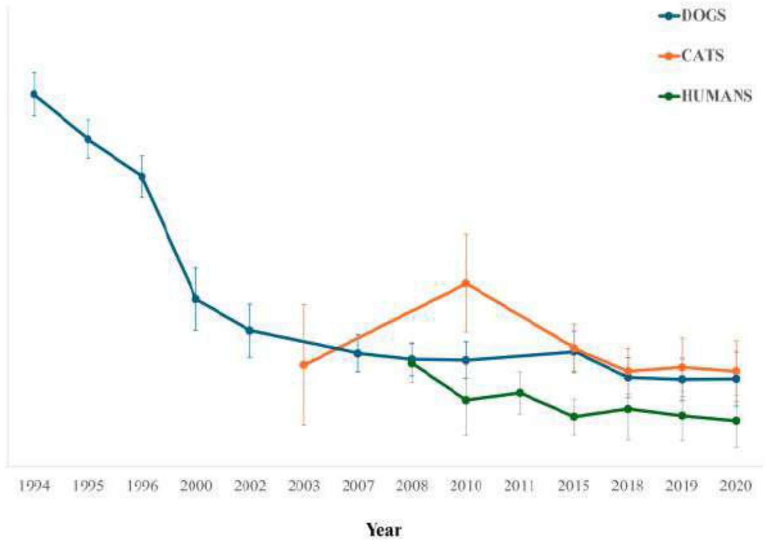
Canine heartworm disease is considered an endemic disease in the European continent (McCall et al., 2008). Over the last decade, *D. immitis* has continued to spread to countries in Eastern and Northeastern Europe, some of which are considered new endemic countries for the disease (Morchón et al., 2022). On the other hand, the prevalence of the disease has continued to increase in southern European countries, traditionally endemic, such as Spain and Portugal (Genchi et al., 2020). In Portugal, an endemic area for the disease, the reported prevalence in the past has remained at similar percentages or even increased in some regions (Cardoso et al., 2012), with prevalence in the north of the country at 2.9%, in the center at 2.4%, and in the south at 5.1% (Maia et al., 2015).

In Spain, the overall prevalence of the disease is 6.25-6.47% throughout the territory (Montoya-Alonso et al., 2020; Montoya-Alonso et al., 2022, Montoya-Alonso et al.,2024). In most autonomous communities, the prevalence does not exceed 10%, except in the Canary Islands (11.58%) and the Balearic Islands (10.87%), with rates of 5-10% in almost all regions and 1-5% in northern communities.

In the Canary Islands, previous research has shown a higher prevalence in the past with a gradual decrease over the years, probably due to prevention campaigns carried out by veterinarians (Montoya-Alonso et al., 2016), resulting in a reduction of up to 30% (Montoya-Alonso et al., 2010). However, the disease has been currently reported in Lanzarote, previously considered free of heartworm (Montoya-Alonso et al., 2017). The prevalence of *Dirofilaria immitis* in dogs on the island of Gran Canaria up to 2020 is 15.81%, while the current seroprevalence in cats is 17.20% (Montoya-Alonso et al., 2024)

Epidemiological data in feline species indicate that its incidence is higher in countries traditionally endemic for the disease, such as Spain, Portugal, and Italy. Cases have also been reported in previously unexpected areas such as Germany and Austria. In Spain, the overall seroprevalence throughout the territory is 9.4% for anti-*D. immitis* antibodies. The southern areas show the highest incidence, such as the Canary Islands (19.2%) and the Balearic Islands (16%), along with Zaragoza (24.4%) and Barcelona (11.47%). Specifically, the Canary Islands have reported a progressive increase in the seroprevalence of the disease, reaching up to 22.9% in 2022 (Montoya-Alonso et al., 2022) **(Figure 1).** Furthermore, several studies correlate feline seropositivity with the incidence of canine heartworm disease (Genchi et al., 2020; Montoya-Alonso et al., 2022), estimating that it may be equivalent to 5-10% of the reported prevalence in dogs in the same area, potentially reaching 20% in certain 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.



CLINICAL SIGNS

The clinical presentation in dogs infected with *D. immitis* can differ widely, ranging from asymptomatic to severe clinical symptoms. Typically, the disease advances slowly, and signs may not become apparent until the parasite has matured into its adult stage. Symptoms can manifest as early as a year after infection but may not become evident until several years later (Kittleson, 1998; McCall et al., 2008b). On the other hand, the appearance of clinical signs depends on the relative parasite load, chronicity of the infection, degree of sedentarism, and the individual host's reaction to the parasite (Dillon et al., 1995; Bowman and Atkins, 2009; Ames and Atkins, 2020).

Clinical signs develop gradually, with the main indicator generally being exercise intolerance, followed by unproductive and chronic cough progressing to moderate to severe dyspnea, prostration, ascites, cachexia, and syncope after exercise (Venco et al., 2005). Other described signs include hemoptysis and/or epistaxis due to the rupture of some pulmonary vessels caused by pulmonary vascular inflammation (Carretón et al., 2012). In advanced cases, pulmonary thromboembolism, right-sided heart failure, vena cava syndrome, and/or death of the animal may occur (Atwell et al., 1988).

During the physical examination, cardiac auscultation may be normal, or a systolic murmur may be detected in the right apical region in cases of tricuspid insufficiency. A diastolic pulmonary murmur may be found in cases of pulmonary valve insufficiency. Pulmonary auscultation may reveal diffuse pulmonary crackles, more evident in caudal lung lobes, although the absence of lung sounds does not rule out pulmonary involvement (Ames and Atkins, 2020).

Other clinical signs in chronic cases include abdominal distension and the presence of jugular pulse due to increased pressure in the right atrium and derived from right congestive heart failure. Also included among these findings are hepatomegaly, splenomegaly, and ascites. Pleural effusion, pericardial effusion, or subcutaneous edema are less frequent signs, the latter being associated with hypoalbuminemia (Ettinger and Feldman, 2009b; Ames and Atkins, 2020). In cases of vena cava syndrome, the lysis of red blood cells produced by parasites lodged in the right atrium leads to the appearance of hemoglobinuria and/or anemia.

The severity of the disease in dogs can be classified based on clinical signs to establish a prognosis and initiate appropriate treatment. Currently, the clinical classification ranges from class 1 to 4, from lower to higher severity. Patients in class I are positive for *D. immitis* but do not show clinical signs. On the other hand, dogs in class 2 present moderate signs, including anemia and proteinuria. Proteinuria is associated with glomerulonephritis associated with chronic antigenic stimulation and immune complex deposition in the glomerulus. Dogs in Class 3 present a combination of moderate to severe clinical signs, radiographic features, and changes in laboratory analyses. Dogs in class 4 are those in which vena cava syndrome develops. These patients typically have elevated pulmonary pressures and high parasite burdens (Miller, 1998; Maxwell et al., 2014).

In the feline species, the most common clinical signs are respiratory and digestive, such as dyspnea, tachypnea, coughing, vomiting, and diarrhea. Other less specific clinical signs that may be found during the physical examination include generalized weakness, weight loss, and anorexia (Pennisi et al., 2020). Conversely, the arrival and death of immature forms of *D. immitis* at the pulmonary level induce heartworm-associated respiratory disease (HARD), leading to a sudden increase in heart rate, blindness, collapse, seizures, and sudden death (Dillon et al., 2014; Dillon et al., 2017b).

Table 1: Classification of symptoms during Heartworm Disease infection.

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 antigens of *D. immitis* is a highly sensitive diagnostic technique. These types of tests are useful in dogs presenting clinical symptoms or in asymptomatic dogs. Presently, there are several commercial tests available with different sensitivity and specificity values (Courtney and Zeng, 2001, Atkins, 2003; Lee et al., 2011). The most used are the ELISA (enzyme-linked immunosorbent assay) technique and immunochromatographic tests, which detect proteins produced in the reproductive tract of adult female of *D. immitis* (Atkins, 2003; Henry et al., 2018). Circulating antigens are detectable only when females reach the adult stage, therefore antigen tests should not be performed earlier than 6 months post-infection (McCall et al., 2008). Several studies have shown that the sensitivity of these types of tests for antigen detection depends on the parasite burden, as well as the sex and age of the parasites (Lee et al., 2011), while specificity ranges between 95%-100% (Courtney, 2001; Atkins, 2003).

Image 1: Commercial test for the detection of *D. immitis* antigens with a positive result.

On the other hand, the inability to detect circulating antigens in dogs and cats infected by *D. immitis* has been ascribed as a consequence of the formation of immune complexes, making the antigen undetectable and yielding false negative results (Little et al., 2018). It has been suggested that heat treatment may disaggregate these complexes and increase the sensitivity of the antigen test without compromising its specificity, although currently, its practice is not recommended in routine screening for suspected infections caused by *D. immitis* (Little et al., 2014; Beall et al., 2017; Little et al., 2018; Murillo et al., 2023). However, other authors report false positive results after subjecting samples to heat treatment in dogs infected with *A. vasorum* and *D. repens* (Ciuca et al., 2016; Venco et al., 2017).

The diagnosis of heartworm disease in cats is relatively more complex compared to dogs, because of infected cats usually have a low parasite burden, with mono-sexual infections being the most common (Genchi et al., 2008, Dillon et al., 2017a). Furthermore, most cases are usually amicrofilaremic (Venco et al., 2015; European Society of Dirofilariosis and Angiostrongylosis, 2017; Pennisi et al., 2020).

In feline species, tests performed using ELISA or immunochromatography for the detection of circulating antigens of adult female *D. immitis* have low sensitivity. Generally, in mono-sexual infections or where there is only one adult female, a false negative result is often obtained in ELISA and immunochromatography antigen tests. Due to this, it is suggested that any negative result not be excluded from the disease (European Society of Dirofilariosis and Angiostrongylosis, 2017).

Image 2: anti-D. immitis test for the detection of *D. immitis* antibodies in feline specie.

Another alternative to antigen detection in feline species is the detection of anti-*D. immitis* antibodies, where exposure to the parasite can be detected at 2 months post-infection regardless of the parasite burden (Prieto et al., 2002). Other authors indicate that serological detection of antibodies is detected from 8 weeks to 5 months post-infection (Litster and Atwell, 2008; Lee and Atkins, 2010).

On the other hand, the test itself indicates exposure to the infection, but does not distinguish between a past or current infection at the time of testing (Dillon et al., 2017). In a follow-up study of naturally infected cats with positive antibody tests, it was found that cats continued to test positive for one to three years after the initial detection (Venco et al., 2011). Therefore, positive antibody results cannot indicate when the infection was acquired or whether the patient is currently infected with immature or adult forms of the parasite. Thus, the persistence of antibodies may reflect a false positive in cats that have already overcome the infection (Berdoulay et al., 2004).

For a positive diagnosis due to adult forms infection, antigen tests are recommended when associated clinical signs are present, although a negative result does not rule out the disease, as this result could be due to the presence of immature forms, low parasite burden, few or no mature females, or the formation of immune complexes that prevent their detection.

Therefore, it is important to use antigen and antibody detection tests in combination to increase diagnostic accuracy in this species, as a negative result for disease detection alone is not conclusive.

MICROFILARIA DETECTION

The detection of circulating microfilariae is a complementary method for the diagnosis of the disease, since between 10-20% of the patients can be amicrofilaremic, due to mono-sexual parasitism, immaturity of the parasites, ectopic location, or destruction of microfilariae due to intermittent preventive treatment or by the host's immune system. (Rawlings et al., 1982; Ettinger and Feldman, 2009b). However, microfilaremia detection is essential in those animals suspected of infection but have obtained a negative antigen test (Velasquez et al., 2014).

There are several tests for microfilariae detection including direct smear, microhematocrit tube test, or modified Knott test, with the latter being the most sensitive for microfilaria detection, as it is a concentration technique that allows the evaluation of microfilaria morphology under the microscope, differentiating infections caused by *D. immitis* from other filaroid nematodes (Knott, 1939; Rojas et al., 2015; Zajac et al., 2021).

Image 5: Blood sample of *D. immitis* microfilariae.

PROPHYLAXIS

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

The use of macrocyclic lactones such as avermectins (ivermectin, abamectin, selamectin) and milbemycins (milbemycin oxime and moxidectin) are the recommended medications for disease prevention. To facilitate their administration, there are different presentations and frequencies of application, such as monthly topical and oral administration, and annual injectable administration (Prichard, 2021). The periodic administration strategy is based on the premise that dogs are constantly exposed to mosquito bites, so the administration of macrocyclic lactones ensures that no live larvae reach the pulmonary arteries, even in cases of delayed administration of the medication (Nolan and Lok, 2012).

The pharmacological effect of macrocyclic lactones during the different stages of the parasite is still not precisely known. However, based on other models such as *Haemonchus contortus* and *Cooperia oncophora*, it is believed to act on glutamate-gated chloride channel receptors and P-glycoprotein, among others (Wolstenholme et al., 2004). These are responsible for regulating the motion and reproduction of the parasite. When the drug binds to these receptors, paralysis of the worm occurs, which proves to be fatal as it facilitates the expulsion of the parasite by the host (Prichard et al., 2001). There are also studies indicating that this class of drugs disrupts the excretory-secretory organ function in larval stages, leading to less effective secretion of immunomodulatory substances by the parasite, making them more susceptible to the host's immunological mechanisms (Moreno et al., 2010).

Lastly, in the feline species, prevention becomes even more important, 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 this disease (Nelson et al., 2005)

TREATMENT

Before initiating a treatment plan, it is necessary to consider several factors influencing the severity of the disease, including the relative parasite burden, chronicity of infection, exercise level of the patient, and individual response to the parasite (Bowman & Atkins, 2009). Sometimes, it is very challenging to determine each of these factors accurately; in many cases, dogs may exhibit symptoms beyond intermittent coughing, indicating chronic disease. Therefore, obtaining a comprehensive patient history and physical examination is essential to rule out even concurrent conditions.

The therapeutic protocol involves treating the various forms in which the parasite and the endosymbiotic bacterium *W. pipientis* can manifest, as well as preventing reinfection and treating associated lesions.

ELIMINATION OF MICROFILARIAE

As previously mentioned, eliminating circulating microfilariae is essential to prevent the disease, but also before starting adulticidal treatment. This is achieved by administering macrocyclic lactones at preventive doses. Administration should be initiated 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 during this time, it allows those parasitic forms not susceptible to macrocyclic lactones to continue their development until they reach pre-adult and adult forms that are sensitive to melarsomine dihydrochloride (Atkins & Miller, 2003; McCall et al., 2004).

TREATMENT AGAINST *WOLBACHIA PIPIENTIS*

Wolbachia pipientis, as previously described, acts as an endosymbiont favoring the parasite's development within the host, so its elimination before initiating adulticidal treatment prevents the inflammatory cascade (Kramer et al., 2005). Doxycycline for 28 days at 10 mg/kg every 12 hours 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 effect (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 remember that doxycycline treatment inhibits parasite embryogenesis and thus reduces 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 includes a total of three injections, with the first dose separated from the second by 30 days, and the second and third doses given on two consecutive days. Administration may be postponed in dogs with severe infections or post-adulticidal treatment complications. This protocol gradually kills adult forms to minimize the risk of pulmonary thromboembolism (Atkins & Miller, 2003). Side effects may occur after melarsomine administration, with the most common being mild swelling and pain at the injection site, reluctance to move due to lumbar pain, depression, panting, anorexia, and vomiting (Maxwell et al., 2014; Ames & Atkins, 2020).

SURGICAL TREATMENT

For patients with vena cava syndrome, there is a surgical alternative involving the extraction of adult worms using fluoroscopy and echocardiography through different existing techniques, such as forceps, loops, or basket or brush-type devices (Yoon et al., 2011; Saunders, 2015; Kim et al., 2023). The success of treatment varies between 50% and 67% because most dogs with vena cava syndrome are hemodynamically unstable before worm extraction, although it is an available option in cases where euthanasia is the only other alternative (Bové et al., 2010).

TREATMENT OF PULMONARY TROMBOEMBOLISM

During the treatment of cardiopulmonary dirofilariasis, the risk of developing thromboembolism is high. This is because after the death of adult parasites, they will fall into the pulmonary vasculature, triggering an almost immediate inflammatory reaction (Sasaki et al., 1992; Carretón et al., 2013). Among the main clinical signs are coughing hemoptysis, local hemorrhages, or disseminated intravascular coagulation (Calvert & Rawlings, 1985; Ames & Atkins, 2020). Treatment in such cases focuses on symptom control using steroids, oxygen therapy, and exercise restriction (Ames et al., 2020). The use of steroids must be justified and initiated in dogs with signs associated with thromboembolism, as improper administration can induce hypercoagulability (Rose et al., 2011; Carretón et al., 2014; Ames et al., 2020).

Image 3 and 4: Lateral-lateral and ventrodorsal radiographic projections of a patient with respiratory distress due to a pulmonary thromboembolism secondary to the death of adult worms.

TREATMENT IN CATS

Management of dirofilariasis in cats involves providing supportive care. Currently, there is no adulticidal treatment available that increases the survival rate in this patient affected by this disease.

In most cases, a conservative approach is preferred, as the infection in this species is usually self-limiting and asymptomatic. Therefore, treatment is not administered to patients who show no clinical signs and have no alterations in pulmonary vasculature and lung parenchyma on radiographic examination. However, periodic monitoring of the patient is necessary, repeating antibody and antigen tests, chest radiography, and echocardiography every 4-6 months.

In cats that may or may not show clinical signs associated with radiological alterations compatible with lung disease, prednisolone at 2 mg/kg administered at decreasing doses over 4 weeks intramuscularly has been found to be effective. When severe clinical signs are present, the patient should be stabilized by administering fluids, intravenous corticosteroids, bronchodilators, and oxygen supplementation.

Regarding adulticidal treatment, eliminating adult parasites is not recommended because melarsomine dihydrochloride is toxic even at low doses, with an efficacy of only 36%. It has been reported that administering ivermectin at a dose of 24 μg/kg monthly for 2 years reduces worm burdens by 65% compared to untreated cats. Treatment with ivermectin is risky because after the parasite's death, the anaphylactic reaction that occurs can jeopardize the patient's life.

The utility of doxycycline in cats infected with *W. pipientis* remains uncertain, as there is no evidence to suggest that it has the same beneficial effect reported in dogs. Furthermore, there is a lack of data regarding the adulticidal effect of the macrocyclic lactone/doxycycline combination in cats. Consequently, doxycycline is not currently recommended as complementary therapy for cats (European Society of Dirofilariosis and Angiostrongylosis, 2017; American Heartworm Society, 2020).

Image 5 and 6: Lateral thoracic radiographs of a 3-year-old cat affected by immature stages of *D. immitis*, demonstrating a diffuse, generalized bronchointerstitial pattern.

CONFIRMATION OF TREATMENT SUCCESS

The antigen test to confirm treatment success should be performed 6 months after the last melarsomine injection. If the test remains positive, it should be repeated after another 2-3 months. It is also necessary to ensure that microfilariae have been eliminated. If the dog has not been treated with macrocyclic lactones and doxycycline (microfilariae are usually eliminated in approximately 3-7 months), the use of a registered microfilaricide is recommended (European Society of Dirofilariosis and Angiostrongylosis, 2017; American Heartworm Society, 2020).

PROGNOSIS

Generally, in cases where the animal shows no symptoms, the prognosis is favorable. Conversely, in patients with severe symptoms and advanced stages of the disease, the prognosis is guarded. Therefore, it is essential to stage the disease before starting treatment, with the aim of assessing the risk of developing thromboembolism. In cases where this risk is very high, modifications to the standard treatment and/or opting not to undergo treatment may be considered (Montoya and Carretón, 2012; Romano et al., 2021). In cases of chronic and severe parasitism, the potential side effects of the disease itself must be considered, such as disseminated intravascular coagulation, pulmonary thromboembolism, right sided heart failure, pulmonary hypertension, among others, some of which may be irreversible once treatment is completed (Tudor et al., 2014; Falcón-Cordón et al., 2019).

SECOND SECTION: THE INVASION AND ITS LESIONS

PATHOPHYSIOLOGY OF PULMONARY HYPERTENSION

PATHOPHYSIOLOGY IN DOGS

The localization of adult parasites in the pulmonary arteries produces key lesions for the subsequent development of the disease, affecting 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 elevation of arterial pressures, causing pulmonary hypertension (Atwell et al., 1988; McCall et al., 2008).

PROLIFERATIVE PULMONARY ENDARTERITIS

The inflammation occurring in the vascular endothelium due to the mechanical action of adult parasites causes anatomical changes within the pulmonary arterial wall and an increase in the size of endothelial cells (Venco and Venzzoni, 2001). These cells can regulate the flow of luminal content into the perivascular tissue, favoring the migration of smooth muscle cells to the vascular media and intima, leading to the formation of villi on the walls of affected arteries, becoming rough and purplish in color (Rawlings et al., 1986; Carretón et al., 2012). These inflammatory mechanisms also result in thickening of the vascular intima, leading to narrowing of the arterial lumen (Furlanello et al., 1998; McCall et al., 2008), increasing the risk of obstruction by embolization of smaller arteries (Kittleson and Kienle, 2000; Kramer et al., 2008; McCall et al., 2008b; González-Miguel et al., 2015) and increasing vascular resistance and pulmonary arterial pressure (Quinn and Williams, 2011a; Tai and Huang, 2013).

Due to the action of endothelial cells, the migration of neutrophils has also been described, along with their adhesion to the endothelial surface and platelet activation in response to endothelial damage, allowing albumin and other blood cells to reach the perivascular space, promoting leukocyte infiltration into the arterial wall (Venco et al., 2014a).

Furthermore, these mechanisms lead to a loss of elasticity and increased tortuosity of damaged arteries, resulting in dilation and increased diameter. Remodeling of the pulmonary arteries begins after the arrival of adult parasites in the peripheral branches of the pulmonary artery, the primary site where the parasite usually settles, and then progresses to proximal segments as the infection becomes chronic (Gómez et al., 1999).

In addition to the endothelial cell response to damage, proliferative pulmonary endarteritis triggered by the parasite itself occurs through the activation of the plasminogen/plasmin system, leading to overproduction of plasmin, which causes degradation of the extracellular matrix and promotes cell migration into the arteries, contributing to the formation of vascular microvilli. Other authors suggest that this alteration of fibrinolytic balance is a survival mechanism through which *D. immitis* could control clot formation in its intravascular habitat, although the key factors on which the benefits or disadvantages of this interaction depend are still unknown (González-Miguel et al., 2015).

Image 7: The image above depicts extensive parasitic infestation by adult forms of *D. immitis* in an adult dog, along with the resulting lesions in the vascular intima of the artery caused by the parasite.

PULMONARY HYPERTENSION

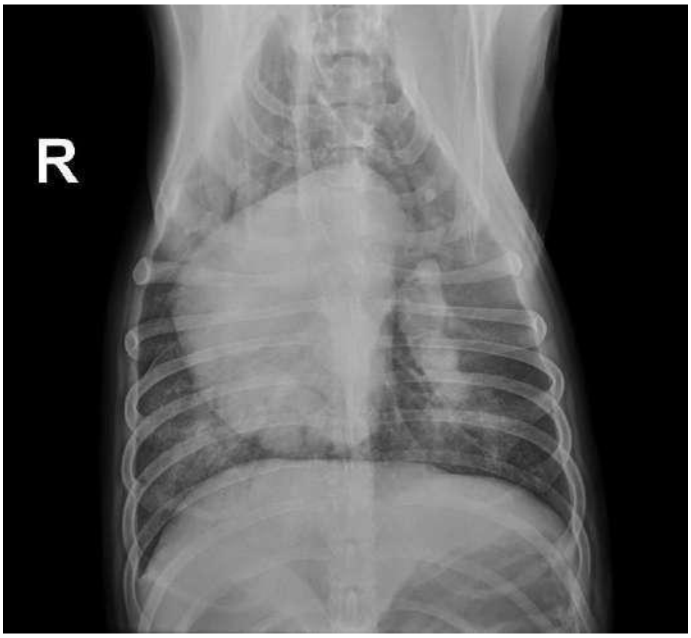
The etiology of pulmonary hypertension (PH) can be primary or secondary and is due to a series of alterations occurring in the pulmonary vasculature, resulting in abnormal and persistent elevation of pulmonary arterial pressure (Serres et al., 2007; Kellihan and Stepien, 2012; Jaffey et al., 2019; Reinero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

In the case of *D. immitis*, PH is classified as precapillary, caused by the adult parasite itself, which increases pulmonary vascular resistance and consequently, increases afterload due to vasoconstriction and vascular remodeling, resulting in reduced vascular lumen, loss of elasticity of pulmonary arteries, and formation of pulmonary thromboembolism (Reinero et al., 2020)

The severity or degree of pulmonary hypertension will be conditioned by the quantity of adult parasites, the duration of infection (chronicity), the host's own reaction to the infection, and the level of physical activity of each patient (Knight et al., 1987; Dillon et al., 1995; McCall et al., 2008a; Atkins et al., 2011). However, some authors indicate that parasite burden does not 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 usually moderate, although the level of pulmonary hypertension may increase up to threefold during exercise, due to increased blood flow required by the right side of the heart (Kittleson et al., 1998).

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

Image 8: The ventrodorsal (VD) view reveals right-sided cardiomegaly, consistent with *D.immitis* infection. Additionally, there is evidence of main pulmonary artery dilation.



As the disease progresses, this pressure overload will cause hypertrophy of the right ventricle (RV), as well as fibrosis of the endocardium and subendocardium. Initially, hypertrophy will not affect the internal diameter of the RV (concentric) (Hoch and Strickland, 2008; Poser and Guglielmini, 2016), but over time, hypertrophy will become eccentric, and the diameter of the RV will increase (Nalije et al., 2013). Chronic pressure overload in the RV leads to increased contractility in early stages, thus preserving systolic function. However, as ventricular remodeling progresses, the ventricle's filling capacity decreases, leading to diastolic dysfunction (Gaynor et al., 2005). Additionally, when the number of adult parasites is excessive, they can migrate and aberrantly locate in the right atrium, right ventricle, and often in the vena cava. This form of blockage is called vena cava syndrome and is 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 RV and right atrium (RA) is multifactorial (Strickland, 1998; Bove et al., 2010). Possible causes include elevated pressures in the pulmonary artery, decreased cardiac output, high parasite burden, alterations in parasite maturation, and/or administration of adulticidal or preventive treatment. Clinically, it is characterized by the onset of acute and severe clinical signs, although in some patients, they may be mild.

On the other hand, regarding the role played by the RA, some authors suggest that initially, the RA increases contractility to facilitate filling of the right ventricle (atrial impulse), but as the disease progresses, it begins to dilate to maintain cardiac output and prevent congestive heart failure on the right side due to chronic pressure overload in the RV (Hoit et al., 1993; Nishikawa et al., 1994). Therefore, the compensatory capacity of the RA is of great importance, as in cases where it does not occur, signs of congestive right heart failure may appear (Laks et al., 1969; Dillon et al., 1995; Gaynor et al., 2005; McCall, 2008).

PULMONARY ALTERATIONS

The pulmonary vasculature constitutes a low-pressure, high-capacity blood storage circuit. Pulmonary arteries possess significant elastic capacity, with highly distensible walls composed of an intima, media, and adventitia layer. The intima, in direct contact with the vascular lumen, consists of a single layer of endothelial cells, collagen, and some fibroblasts. The media is thicker and comprised of elastin, collagen, and smooth muscle cells. The adventitia primarily comprises 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, leading to perivascular edema and inflammation and, in advanced stages, irreversible fibrosis (Rawlings et al., 1986). Severe cases may also feature irregular lesions and overgrowths resembling villi in the vascular intima, invading the arterial lumen. Occasionally, vascular intimal wall necrosis may also be observed (Quinn and Williams, 2011a). All these alterations impair gas exchange, contributing to increased resistance.

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

Thus, 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 cases, 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 dirofilariasis (Shibata et al., 2000).

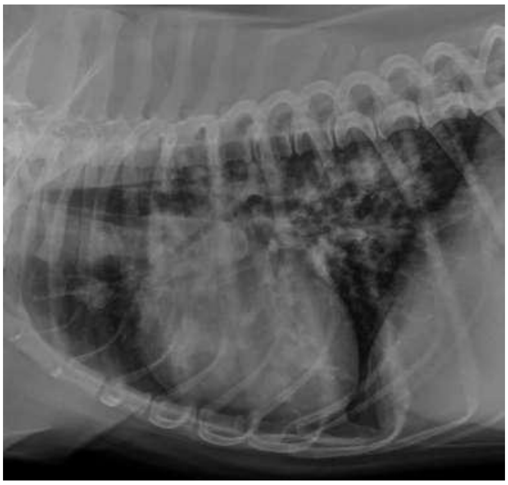
Other possible pulmonary lesions described in dirofilariasis cases include pulmonary eosinophilic infiltration syndrome (Confer et al., 1983; Atwell and Tarish, 1995; Reinero et al., 2019). Specifically, this occurs in cases of hypersensitization to antigens produced by microfilariae, where they are neutralized and immediately destroyed in the pulmonary circulation (Calvert and Losonky, 1985). Microfilariae detection facilitates leukocyte and eosinophil influx, generating granulomatous inflammation (Confert et al., 1983; Bowman and Atkins, 2009).

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

PULMONARY THROMBOEMBOLISM

Pulmonary thromboembolism (PTE) occurs following the obstruction of one or more pulmonary vessels secondary to the death of adult parasites. Cardiopulmonary dirofilariasis is among the causal agents of PTE due to alterations in one of Virchow's triad factors: hypercoagulability, blood flow stasis, or endothelial injury. In the case of *D. immitis*, alterations affect the endothelial integrity of the arterial wall and lead to increased blood hypercoagulability (Keith et al., 1983; LaRue et al., 1990). All of this favors the formation of villi in the vascular intima and perivascular edema formation (Schaub and Rawlings, 1980).

Figure 9 and 10: The lateral radiographs reveal a pattern consistent with pulmonary thromboembolism in a dog infected with *Dirofilaria immitis*. The images demonstrate an alveolar infiltration pattern characterized by regions of opacity indicative of secondary pulmonary congestion. Additionally, multiple pulmonary nodules or lesions are observed, suggesting ischemia in the affected lung regions.



Moreover, parasite fragments are subjected to the host's immune system, becoming partially incorporated into the arterial wall, where a large amount of connective tissue will form as scar tissue (Atkins et al., 2005). Additionally, parasites will occlude smaller pulmonary vessels, with the caudal lobes being the most affected, resulting in reduced or interrupted blood supply, leading to 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

Dirofilariasis manifests differently in feline species, with cats being much less immunologically tolerant to *D. immitis* infection. Additionally, there are two different forms of the disease presentation: one caused by immature parasites and the other by chronic disease caused by adult forms.

Generally, in feline species, the parasitic load is low, with pathological changes appearing early and severely, potentially leading to mortality or sudden death 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 cases where the parasite reaches its adult form, cats may be asymptomatic. It is believed that mature adult parasites secrete mediators that regulate the activity of polymorphonuclear cells, resulting in an anti-inflammatory effect that minimizes clinical signs.

The inflammatory response at the pulmonary level becomes evident once immature worms reach the pulmonary vessels, around 90 days post-infection. In most cases, these immature forms are destroyed by the host between 3-4 months post-infection, preventing their development into adult forms (Dillon et al., 2007; 2014). Unlike dogs, cats have pulmonary intravascular macrophages, which can be modulated by parasitic products and may contribute to the distinct pathophysiological effects in cats compared to dogs (Dillon et al., 2008).

Moreover, when there is early death of larval forms of the parasite, severe acute lesions occur, such as pulmonary thromboembolism and eosinophilic inflammatory pneumonitis, causing the so-called HARD (Atkins and Litster, 2006; Garcia-Guasch et al., 2013). In these patients, pulmonary disease primarily affects the bronchial, interstitial, and pulmonary artery levels (Dillon et al., 2007). After this initial infection, cats with HARD develop antibodies against *D. immitis*, although there are cases where antibodies do not develop up to 50% (Dillon et al., 2007). Therefore, the development of HARD is conditioned by the presence of immature worms (Browne et al., 2005).

Additionally, 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, as well as involvement of small airways and alveolar infiltration. Interlobar pulmonary branches are primarily affected, with thickening of their walls (Blagburn et al., 2007). There is also bronchial hyperreactivity and/or inflammation caused by the infection. Bronchial hyperreactivity is characterized by excessive and reversible smooth muscle contraction (bronchoconstriction) in response to stimuli and is a common sequel to inflammation and bronchial damage (Liu et al., 2006).

Image 11 and 12: Lateral and ventrodorsal view of a cat infected with *D.immitis*, showing a diffuse bronchointerstitial pulmonary pattern and pulmonary hyperinflation

THIRD SECTION: EXPOSING THE PARASITE

RADIOLOGICAL STUDY OF PULMONARY HYPERTENSION.

In human medicine, the assessment of the usefulness of thoracic radiology in diagnosing and staging cardiopulmonary diseases compared to other imaging techniques has been extensively studied (Fisher et al., 2010; Pagnamenta et al., 2015). Additionally, the association between present clinical signs and radiological alterations is fundamental in issuing a diagnosis (Woznitza et al., 2018).

On the other hand, in veterinary medicine, thoracic radiography is a widely accessible and commonly used tool in the daily basis clinical practice of small animals (Thrall, 2018). Furthermore, it is a cost-effective and useful diagnostic technique for the initial investigation of a wide range of pulmonary and cardiac diseases in dogs (Rudorf et al., 2008). Its interpretation can sometimes be challenging as the visible structures on radiographs vary depending on the size, age, breed, and sex of the animal (Mai et al., 2008; Thrall, 2018). This diagnostic technique, used based on a clinical history compatible with cardiorespiratory disease, is essential in orienting the diagnosis (Pinto and Brunese, 2010; Arruda et al., 2023), although some authors indicate that the operator's knowledge of clinical signs may negatively influence its interpretation (Small, 2021).

There are numerous diseases in dogs and cats that result in cardiac and pulmonary alterations, for which thoracic radiography can provide valuable insights into disease progression (Atkins et al., 2009; Borgarelli et al., 2015; Malcolm et al., 2018). Additionally, thoracic x-rays, when employed in conjunction with other diagnostic techniques, demonstrate a high correlation in diagnosing certain cardiorespiratory pathologies. These include 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 represents 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 PH 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 PH 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 PH 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).

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

In the case of dogs affected by *D. immitis*, the use of thoracic radiology has proven to be useful in assessing the extent of the disease and determining if the patient is suitable for adulticidal treatment (Atkins, 2010; Tudor et al., 2014; Vetter et al., 2023). Specifically, during the disease, only 25-30% of affected dogs do not show radiological alterations (Polizopoulou et al., 2000; Losonsky, 1983).

This coincides with other authors who point out that thoracic radiography clearly detects these types of abnormalities, with nonspecific alterations detected in 98% of the studied patients, with cardiomegaly being the most frequently observed in 84% of the animals. Additionally, 31% of dogs showed an increase in the pulmonary artery, although no significant differences were obtained between radiological findings and the severity of pulmonary hypertension obtained echocardiographically (Johnson & Orton, 1999).

RADIOLOGICAL SIGNS OF PULMONARY HYPERTENSION

CARDIAC ALTERATIONS.

Radiological evidence suggestive of PH includes right-sided cardiac chamber enlargement and pulmonary artery dilation. Right-sided cardiomegaly is characterized by rounding of the cranial and right lateral margins of the cardiac silhouette, with caudal displacement to the left of the cardiac apex, giving an appearance of an inverted D-shaped cardiac structure on a ventrodorsal/dorsoventral projection (Adams et al., 2017; Bahr, 2017). However, in the feline species, this finding is unusual, with generalized cardiomegaly being more common (Johnson and Hansson, 2013). This may be due to the rarity of PH and right ventricular hypertrophy in this species (Small et al., 2008; Venco et al., 2015).

There are several measures to determine the presence of cardiomegaly, such as the Manubrium Heart Score (MHS), Heart to Single Vertebral Ratio (HSVR), and Thoracic Inlet Heart Score (TIHS), which have proven to be useful in studying the cardiac silhouette (Mostafa & Berry, 2017; Costanza et al., 2023; Marbella et al., 2023).

Although the most widely used method for determining cardiomegaly is through the vertebral heart score (VHS) or Buchanan index, taking on a lateral thoracic radiograph the longitudinal cardiac axis (from the base of the carina to the cardiac apex) and the short cardiac axis (maximum axis perpendicular to the longitudinal axis in the middle third). These values are transferred to the vertebral column, counting from the anterior facet of the fourth thoracic vertebrae (T4). The number of thoracic vertebral bodies corresponding to both axes is determined and added together (Buchanan, 2000). However, there are cases where left-sided cardiomegaly is not evident, with cardiac chambers widening in relation to their height. This alteration can be checked through the cardiac proportion of 3/5 to 2/5, by drawing a line parallel from the carina to the apex of the heart. Approximately 3/5 of the cardiac silhouette area should be cranial to this line, while the remaining 2/5 of the area should be caudal to this line under normal conditions. If the percentage of cranial cardiac silhouette exceeds 3/5, right-sided cardiomegaly is present (Suter, 1984).

Some authors point out that the study of cardiac remodeling may sometimes provide subjective information about the presence and severity of enlargement of cardiac chambers and blood vessels, especially in cases of moderate to severe left-sided cardiomegaly, due to the distortion of cardiac chambers, giving a false impression of right-sided cardiomegaly (Carlsson et al., 2009; Huget et al., 2021). Other authors suggest that these associated alterations such as inverted D shape, main pulmonary artery dilation, and signs of congestive heart failure do not present in the same way in large dogs and small dogs, so thoracic radiography may underestimate the severity of the disease in small dogs infected with *D. immitis*, so they will need an echocardiographic study (So-Young et al., 2019).

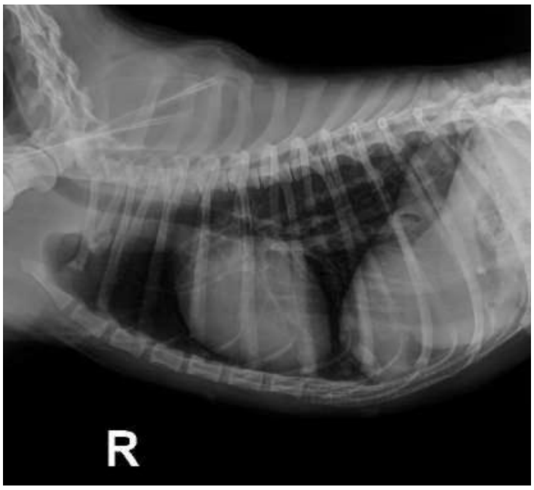
Additionally, studies suggest that the Vertebral Heart Score (VHS) can vary depending on the position used in the lateral-lateral projection, with a higher value observed in the right lateral position in healthy dogs (Greco et al., 2008).

Image 13: Dorsoventral projection of Rumi, a 2-year-old canine patient infected with *D. immitis*, exhibiting a parasitic load of 3/4 and dilation of the right cardiac chambers with the classical D-inverted shape.

In cats, the manifestation of congestive heart failure and pulmonary hypertension signs, such as left ventricular or left atrial hypertrophy, is infrequent. As a result, these radiological alterations are typically not evident. However, a loss of collagen within the right ventricular myocardium has been evidenced. This collagen depletion 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).

On the other hand, radiological detection of pulmonary artery dilation appears as an opaque soft tissue density prominence that merges with the cardiac silhouette. Quantitatively, correct evaluation 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 indicates pulmonary artery dilation (Thrall & Losonsky, 1976). Another quantitative technique for reliably measuring the pulmonary artery diameter is by comparing it to the width of the ninth rib in a ventrodorsal/dorsoventral projection. A ratio greater than 1:1 is suggestive of pulmonary artery dilation. In cases of chronic and severe PH, pulmonary vascular tortuosity may also be detected (Bahr, 2017; Reinero et al., 2020).

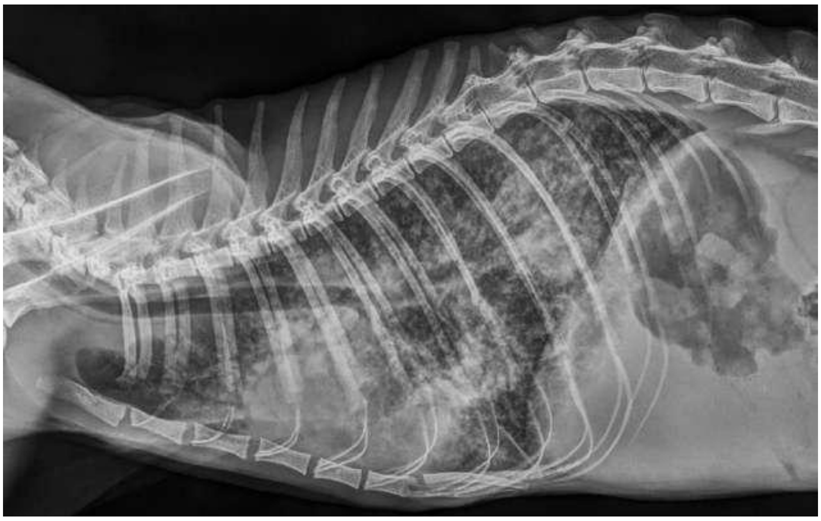
Image 14 and 15: Radiography in dorsoventral and lateral-lateral projections revealing an enlargement of the cardiac silhouette.



PULMONARY PARENCHYMAL ABNORMALITIES

The most frequent pulmonary alterations associated with *D. immitis* infection in dogs are triggered by increased pressure, resulting in enlargement and increased tortuosity of the intralobar and interlobar peripheral branches of the pulmonary arteries. Lesions in the lung parenchyma primarily affect the caudal lobes initially, spreading to the rest as the disease progresses (Calvert and Rawlings, 1988; Rawlings, 1986; Bowman and Atkins, 2009).

Image 16: Lateral-lateral radiograph of a canine patient with acute respiratory distress showing lesions consistent with HARD disease.



In dogs with chronic infection, irreversible mineral deposits occur in 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). Furthermore, in patients with pulmonary thromboembolism, areas of increased soft tissue opacity and/or hypovascularized lucid regions are identified, primarily with a peripheral distribution (Bahr, 2017).

In cats, pulmonary alterations are more evident, as *D. immitis* infection in this species induces a greater inflammatory response in the lung parenchyma. Interstitial inflammation, along with hypertrophy and hyperplasia of type II pneumocytes, along with proliferation of smooth muscle cells, leads to fibrosis and emphysema of the lung parenchyma. Pneumonitis is characterized by the presence of eosinophils, macrophages, plasma cells, mast cells, and fibroblasts (eosinophilic pneumonitis) (Maia et al., 2011; Dillon et al., 2014).

RADIOLOGICAL AND ECHOCARDIOGRAPHIC ASSOCIATION IN HEARTWORM DISEASE.

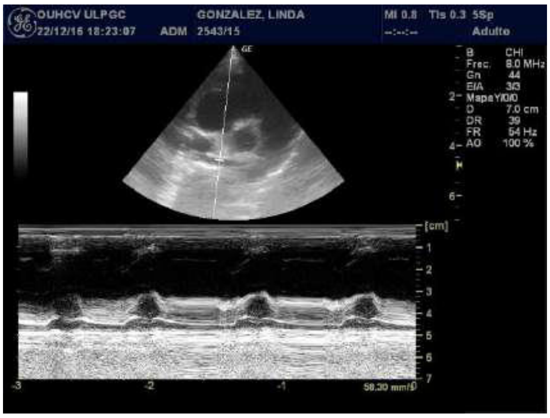
Thoracic radiography has proven to be very useful in dogs with heartworm disease, detecting radiographic abnormalities in up to 74% of cases (Tudor et al., 2014). In the study of PH, thoracic radiography is used in combination with other diagnostic techniques to accurately determine the degree of PH, with echocardiography being the most used. In the case of PH caused by *D. immitis*, the evaluation of the Right Pulmonary Artery Distensibility Index (RPADi) is a validated parameter to estimate the presence and severity of PH in dogs.

Currently, there are several different published methods for obtaining RPAD. Among them is the method according to Venco, in which the diameter of the right pulmonary artery is measured 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). On the other hand, the modified Venco method obtains the measurement from trailing edge to leading edge (te-le).

The third method, the classic Visser method, uses measurements of the right pulmonary artery from edge to edge (le-le), using a right parasternal short-axis view in B-mode (Visser et al., 2018). Additionally, there is also the modified Visser method, where from the same view, the measurement of the pulmonary artery is taken from trailing edge to leading edge (te-le). Finally, another alternative measure proposed by Visser is obtained from the M-mode image, measuring diameters using the edge-to-edge method. In addition to these techniques, other hybrid methods have been published, such as the ratio of the pulmonary vein to the right pulmonary artery, which have been shown to be useful in evaluating PH (Roels et al., 2019; Visser et al., 2020).

The main difference between obtaining the image in B-mode and M-mode is that, in principle, the M-mode method could be superior in the evaluation of RPAD due to more precise delineation of the point where the diameter of the pulmonary artery should be measured in systole and diastole, and because of better temporal resolution compared to B-mode (Basile et al., 2023).

Image 17 and 18: Echocardiographic images showcasing the diverse methodologies for pulmonary hypertension determination. Firstly, the technique proposed by Venco et al., 2014 utilizing M-mode, followed by the approach established by Visser et al., 2016 employing B-mode.



RPAD values below 29% according to the methodology applied by Venco are considered to indicate mild pulmonary hypertension. On the other hand, Visser establishes that pulmonary hypertension is considered when this index is below 34.6%.

These measures have been used together with thoracic radiography for staging and monitoring PH. Echocardiography is employed when radiological evidence of tortuous and/or dilated pulmonary arteries is present, along with compatible pulmonary alterations such as localized or diffuse alveolar infiltration and a prominence in the region of the pulmonary trunk or right-sided cardiac enlargement (Reinero et al., 2020).

However, a comparative study between the severity of radiographic abnormalities and the severity of PH at the echocardiographic level during heartworm disease has not been conducted.

FOURTH SECTION: THE AIM AND THE OUTCOMES

OBJETIVES

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

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 PH in infected dogs by comparing the Right Pulmonary Artery Distensibility (RPAD) Index before and after adulticide treatment. The goal is to assess the persistence of PH in hypertensive dogs over time by monitoring changes in VHS, CrPA/R4, and CdPA/R9 ratios, emphasizing the importance of radiographic evaluation for the long-term management of the disease.

Identify Radiographic Abnormalities in Cats Infected with Immature Forms of *D. immitis*: This objective centers on evaluating the radiographic alterations in cats infected with immature forms of *D. immitis*. The focus is on characterizing changes in the pulmonary artery and the present pulmonary patterns using measurements such as CrPA/R4 and CdPA/R9 ratios and central venous catheter (CVC) metrics to identify vascular damage and correlate the radiographic findings with the presence of clinical signs of heartworm-associated respiratory disease (HARD) caused by *D. immitis*

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

Soraya Falcón-Cordón [1], Yaiza Falcón-Cordón [1], Alicia Caro-Vadillo [2], Noelia Costa-Rodríguez [1], José Alberto Montoya-Alonso [1,*] and Elena Carretón [1]

[1] Internal Medicine, Veterinary Medicine and Therapeutic Research Group, Faculty of Veterinary Medicine, Research Institute of Biomedical and Health Sciences (IUIBS), Universidad de Las Palmas de Gran Canaria (ULPGC), 35016 Las Palmas de Gran Canaria, Spain; soraya.falcon@ulpgc.es (S.F.-C.); yaiza.falcon@ulpgc.es (Y.F.-C.); noelia.costa@ulpgc.es (N.C.-R.); elena.carreton@ulpgc.es (E.C.)
[2] Department of Animal Medicine and Surgery, Faculty of Veterinary Medicine, Complutense University, 28040 Madrid, Spain; aliciac@vet.ucm.es
* Correspondence: alberto.montoya@ulpgc.es

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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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.

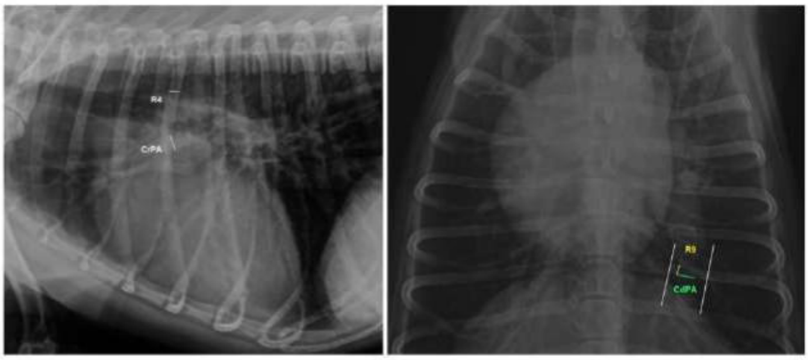
In fact, there are studies that have characterised the association of radiological and echocardiographic findings in dogs with PH [10,11], but there are not as many studies performed in dogs with heartworm disease. Given that PH is a common and serious condition in this pathology and given the unique 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 $\pm$ 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.

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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.

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

Soraya Falcón-Cordón, Yaiza Falcón-Cordón, Noelia Costa-Rodríguez, Jorge Isidoro Matos, José Alberto Montoya-Alonso * and Elena Carretón

Internal Medicine, Veterinary Medicine and Therapeutic Research Group, Faculty of Veterinary Medicine, Research Institute of Biomedical and Health Sciences (IUIBS), Universidad de Las Palmas de Gran Canaria (ULPGC), 35016 Las Palmas de Gran Canaria, Spain; soraya.falcon@ulpgc.es (S.F.-C.); yaiza.falcon@ulpgc.es (Y.F.-C.); noelia.costa@ulpgc.es (N.C.-R.); jorge.matos@ulpgc.es (J.I.M.); elena.carreton@ulpgc.es (E.C.)

* Correspondence: alberto.montoya@ulpgc.es

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

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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-

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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 Day 0–Day 270 Day 90–Day 270	0.01108686 * 0.37772975 0.14238119
VHS	Day 0–Day 90 Day 0–Day 270	0.00137051 * 0.00027207 *
CrPA/R4	Day 90–Day 270 Day 0–Day 90 Day 0–Day 270 Day 90–Day 270	0.05733536 0.16833147 0.2023426 0.36260403
CdPA/R9	Day 0–Day 90 Day 0–Day 270 Day 90–Day 270	0.16833147 0.22349953 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.

[Fecha]

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.

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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.

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animals

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Article

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

Soraya Falcón-Cordón [1], Yaiza Falcón-Cordón [1], Sara Nieves García-Rodríguez [1], Noelia Costa-Rodríguez [1], Daniel Julio Vera-Rodríguez [1], José Alberto Montoya-Alonso [1]* and Elena Carretón [1]

[1] Internal Medicine, Veterinary Medicine and Therapeutic Research Group, Faculty of Veterinary Medicine, Research Institute of Biomedical and Health Sciences (IUIBS), Universidad de Las Palmas de Gran Canaria (ULPGC), 35016 Las Palmas de Gran Canaria, Spain.

* Correspondence: alberto.montoya@ulpgc.es

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 (n=50), asymptomatic and seropositive to *D. immitis* antibodies (n=30) and seropositive and cats with clinical signs (n=43). Different radiographic measurements were assessed including VHS, 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 VHS, demonstrating enlarged vasculature in cats with *D. immitis*. Moreover, 62.8% of the cats with clinical signs showed a marked bronchointersticial pattern, while asymptomatic cats mainly (33.3%) had mild bronchointersticial 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 and high risk of heartworm-associated respiratory disease (HARD) (Group C). Radiographic measurements and lung parenchymal abnormalities were analyzed and compared across 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 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

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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]. 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 [4,5,6].

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 [11,12,13].

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, it has been described an enlargement of the main and peripheral pulmonary arteries, characterized by the loss of conical shape, tortuosity, and truncation of the caudal lobar branches. Additionally, parenchymal alterations are commonly observed, with diffuse or focal broncho-interstitial patterns detectable on radiographs [10,15,16,17]. However, these lesions are not patognomonic and are similar to the lesions found in other diseases, as infections by *Toxocara cati, Aerostrongylus spp.*, asthma or allergic bronchitis [10,18,19,20]. In most cases, these alterations are accompanied by clinical signs such as coughing or dyspnea, among others [5,10,16]. In any case, the radiological study, used in combination with 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 histopathological lesions mainly focused on the main pulmonary artery, arterioles, and bronchial interstitial changes; such as bronchiolar lesions, 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 that 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 which 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 animal 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 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) were also carried out and any cat that tested positive was neither included in 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, 1 Maine Coon 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-minute 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 < 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*

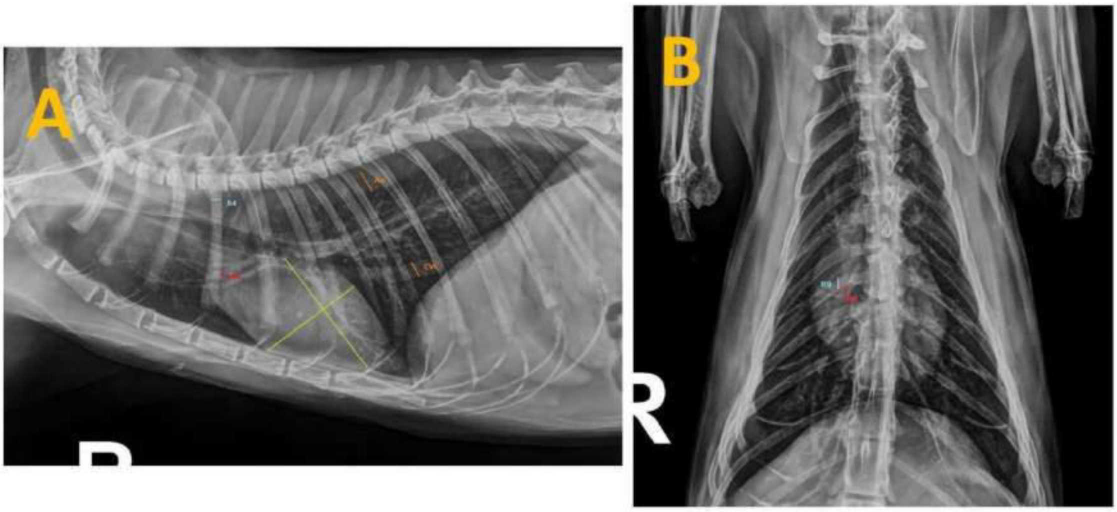
Echocardiograms were conducted on every cat to confirm the presence or absence of adult worms, as well as to exclude any other concurrent diseases. The cats were positioned on their right side with the transducer positioned in the third intercostal space to check for worms in the pulmonary arteries and right 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, 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), and the right cranial pulmonary artery (CrPA) (in red). (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 the R4 [27,28,29].The measurement of the diameter of the descending aorta (Ao) at the same intercostal space as the CVC was done 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 the R4 in the laterolateral projection and the diameter of R4 at a point just distal to the spine. Moreover, in the dorsoventral projections it was measured the distal and left sides of the summation shadow created by the right caudal pulmonary artery (CdPA) with the R9. Finally, CrPA/R4 and CdPA/R9 ratios were calculated from these measurements [30].

In addition, quantitative evaluation of shape and tortuosity of lung vasculature in both radiographic projections was assessed. 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, Siegel Tukey test was performed to verify the variability of variances between groups. The Chi square test was used to assess the association between categorical variables. The non-parametric test of U Mann Whitney was performed to determine differences between groups for all recruited measurements. In all cases, a p value < 0.05 was determined as significant. Continuous variables were expressed as median ± 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 de 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 3 groups: Group A (n=50) consisted of cats with no clinical signs and seronegative for anti-*D. immitis* antibodies, Group B (n=30) consisted of cats seropositive to *D. immitis* but asymptomatic and Group C (n=43) comprised seropositive animals with *D. immitis*, exhibiting clinical signs compatible with 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.

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.

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%

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) 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 Group 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).

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: cats asymptomatic seropositive to anti-*Dirofilaria immitis* antibodies; Group C: cats with clinical signs and 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 seroposisitive 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	3.0545E-21***	0.74419009	Statistically significant difference between seronegative and seroposisitive 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	5.02886E-07***	0.44459144	Statistically significant difference between seronegative and seroposisitive cats
	Group B	1.16±0.14	0.00349851***	0.31565838	Statistically significant difference between group B and C
	Group C	1.35±0.25			
CVC/R4	**Group A**	1.62±0.15	1.2276E-21***	0.74896233	Statistically significant difference between seronegative and seroposisitive cats
	Group B	2.41±0.61	0.021834542*	0.23490465	Statistically significant difference between group B and C
	Group C	2.62±0.47			
CdPA/R9	**Group A**	0.79±0.05	8.7566E-22***	0.75130749	Statistically significant difference between seronegative and seroposisitive cats
	Group B	1.23±0.31	0.017873607*	0.24919937	Statistically significant difference between group B and C
	Group C	1.36±0.34			

***Correlation is significant at 0,5% (p<0,005); ** Correlation is significant at 1% level (p<0,01);.*Correlation is significant at 5% level (p<0,05); ns Correlation is not significant.

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 CVC and VHS (Table 3).

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

[Fecha]

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); *Correlation is significant at 5% level (p<0,05); 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 mild bronchointerstitial (33.3%; 10/30), while in Group C, a marked bronchointerstitial pattern was observed in 62.8% (27/43). 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: cats asymptomatic seropositive to anti-*Dirofilaria immitis* antibodies; Group C: cats with clinical signs and 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 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 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 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 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 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 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 heart disease. Moreover, the echocardiographic exam carried out in the studied cats showed no evidences of right heart disease. Further research is necessary to clarify the relationship between the CVC/Ao ratio and right 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 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 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 part 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 to find certain arterial abnormalities 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 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 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 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 made 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 have 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 indicate the presence of vascular and parenchymal abnormalities in cats with a high likelihood of being infected by immature *D. immitis* parasites, particularly in those exhibiting clinical signs and, therefore, suffering HARD. Consistent with histopathological findings from other authors [6,9,10,23], this may provide evidence of early vascular damage caused by this parasite. Given the challenges in diagnosing infections by immature *D. immitis* worms in cats, the study of these radiographic measurements could serve as a valuable diagnostic tool for veterinary clinicians when HARD is highly suspected. While previous studies have shown that heartworm infection cannot be entirely ruled out in cats with normal thoracic radiographs [3,10], the results of the present study support the diagnostic suspicion of HARD in cats with compatible clinical signs and radiographic findings.

Author Contributions: JAMA and EC designed the study. SFC, YFC and EC wrote the manuscript. SFC, YFC, SNGR, DVR and NCR 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.

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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 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.

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CONCLUSIONS

1. Thoracic radiography is an accessible and widely available imaging technique in daily clinical practice. Specifically, during *D. immitis* infection, certain radiological parameters (CrPA/R4 and CdPA/R9) have shown an acceptable correlation in determining the presence or absence of pulmonary hypertension through echocardiography. Therefore, these values may be useful in the diagnosis and monitoring of the disease in dogs infected with *D. immitis*.

2. The assessment of VHS, CrPA/R4, and CdPA/R9 ratios through radiographic imaging is valuable for detecting and monitoring pulmonary hypertension (PH) in dogs infected with *D. immitis* both before and after adulticide treatment. These indicators show clear differences between dogs with and without PH, and they tend to worsen in those already exhibiting signs of PH after treatment, highlighting the importance of ongoing monitoring.

3. In cats, thoracic radiographic studies prove to be a valuable diagnostic tool, along with the presence or absence of clinical signs, for guiding the diagnosis of feline dirofilariasis by utilizing important parameters such as the CVC/Ao, CVC/R4, and CdPA/R9 ratios. Additionally, the worsening of pulmonary patterns positively correlates with the presence of clinical signs in cats with HARD. These findings underscore the importance of radiographic evaluation in veterinary clinical practice, enabling more accurate diagnosis and better treatment guidance for cats suspected of infection with *D. immitis*.

SIMPLE SUMMARY

The nematode *D. immitis* primarily affects domestic and wild carnivores. This parasite resides in the pulmonary arteries and the right ventricle of the heart, being transmitted mainly by mosquitoes of the Culex, Aedes, and Anopheles genera. Infection is often asymptomatic in dogs but can be fatal if not treated promptly. Clinical signs may include chronic cough, exercise intolerance, and weight loss. In cats, most cases are asymptomatic; however, aberrant migration of parasites can lead to sudden death (Lee and Atkins, 2010). The spread of this disease is linked to the movement of infected animals and climate change, which favors the survival of mosquitoes (Anvari et al., 2019a). Prevalence in Europe has increased, with Eastern and Southern countries like Spain and Portugal reporting rates ranging from 2.4% to 11.58% (Genchi et al., 2020; Montoya-Alonso et al., 2020). In recent years, the Canary Islands have seen a rise in seroprevalence in cats, reaching up to 22.9% in 2022 (Montoya-Alonso et al., 2022). The disease can lead to pulmonary hypertension and heart failure in dogs, while in cats, clinical signs are usually respiratory and gastrointestinal (Pennisi et al., 2020).

Diagnosis is primarily based on the detection of circulating antigens of *D. immitis*, which is a highly sensitive diagnostic technique. These antigen tests are useful in dogs presenting clinical signs or in asymptomatic dogs. The most commonly used methods include the ELISA and immunochromatographic tests, which detect proteins produced in the reproductive tract of adult female *D. immitis* (Atkins, 2003; Henry et al., 2018).

The pathophysiology of pulmonary hypertension due to *D. immitis* infection involves inflammation of the vascular endothelium caused by the mechanical action of adult parasites. This inflammation leads to structural changes in the pulmonary arteries, including thickening of the vascular intima, which narrows the arterial lumen and increases vascular resistance (Furlanello et al., 1998; McCall et al., 2008). Furthermore, inflammatory processes promote the migration and infiltration of leukocytes into the arterial walls, exacerbating vascular damage and contributing to the progression of pulmonary hypertension (Venco et al., 2014a).

However, the gold standard diagnostic technique for determining the presence of pulmonary hypertension is echocardiography, specifically utilizing the RPAD Index. Additionally, thoracic radiography is an accessible and widely available imaging technique in daily clinical practice. In dogs infected with *D. immitis*, the assessment of VHS, CrPA/R4, and CdPA/R9 ratios is useful for detecting and monitoring pulmonary hypertension. These indicators reveal clear differences between dogs with and without pulmonary hypertension and underscore the importance of ongoing monitoring, especially in those exhibiting clinical signs. In cats, parameters such as the CVC/Ao, CVC/R4, and CdPA/R9 ratios have proven to be valuable diagnostic tools that aid in guiding the diagnosis of feline dirofilariasis. Additionally, the worsening of pulmonary patterns in cats correlates positively with the presence of clinical signs in those with HARD, emphasizing the role of radiographic evaluation in veterinary practice for accurate diagnosis and treatment guidance.

Finally, heartworm disease poses a growing public health and veterinary issue in Europe, with serious implications for the health of dogs and cats. Ongoing research is essential to better understand its epidemiology and improve diagnostic strategies.

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SCIENTIFIC CONTRIBUTIONS

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Q1 scientific publications

1. Henríquez-Hernández LA, Carretón E, Camacho M, Montoya-Alonso JA, Boada LD, Valerón PF, Falcón-Cordón Y, Falcón-Cordón S, Almeida-González M, Zumbado M, Luzardo OP. The heartworm (Dirofilaria immitis) seems to be able to metabolize organochlorine pesticides and polychlorinated biphenyls: A case-control study in dogs. Sci Total Environ. 2017 Jan 1;575:1445-1452. doi: 10.1016/j.scitotenv.2016.09.236. Epub 2016 Oct 14. PMID: 27751690.

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