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Research paper

Pulmonary hypertension in dogs with heartworm before and after the adulticide protocol recommended by the American Heartworm Society

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ABSTRACT

Pulmonary hypertension (pH) is a frequent and severe phenomenon in heartworm disease (*Dirofilaria immitis*). There is a lack of studies assessing the evolution of the proliferative endarteritis and pH caused by *D. immitis* after the death of the parasites, so this study evaluated the influence that the elimination of the worms exerts over the pulmonary pressure and therefore evolution of the endarteritis, through the evaluation of the Right Pulmonary Artery Distensibility (RPAD) Index and other echocardiographic measurements in 2D mode, M-mode and Doppler echocardiography in 34 dogs naturally infected by *D. immitis* on day 0, and one month after the last adulticide dose (day 120). pH, based on the determination of the RPAD Index, was present in 68% of the dogs (n = 23) on day 0 and on day 120. No significant differences were observed between the RPAD Index between the two measurements, and only significant differences were found in pulmonary deceleration time, ejection time, and left ventricular internal diameter in telediastole when measurements from day 0 and day 120 were compared. There was not any worsening in the development of pH after the elimination of the parasites, independently of the parasite burden. During the adulticide treatment, the death of the worms causes thromboembolism and tends to worsen the vascular damage and presence of pH . It seems that following the adulticide protocol recommended by the American Heartworm Society with the previous elimination of *Wolbachia* and reduction of microfilariae followed by the stepped death of the worms did not cause a significant aggravation of the pulmonary damage of the treated dogs. Neither is present any significant improvement in the RPAD Index on day 120; probably, more time is needed before appreciating some positive changes after the elimination of the worms and *Wolbachia* from the vasculature and further studies are necessary.
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1. Introduction

It has been previously demonstrated that heartworms (*Dirofilaria immitis*) cause proliferative endarteritis of the pulmonary arteries, one of the main pathogenic mechanisms of cardiopulmonary dirofilariosis (McCall et al., 2008). This endarteritis reduces the arterial lumen, the smaller arteries become obstructed because of embolization, and the arteries lose elasticity. The reduced gauge and compliance of the arteries chronically develops pulmonary hypertension (pH) which, if not treated correctly, can produce right sided-congestive heart failure (McCall et al., 2008; Simón et al., 2012). This series of events are especially true in active dogs independently of the worm burden (Dillon et al., 1995). Other factors, as proteins secreted by the parasites and endogenous molecules, may contribute to the vascular endarteritis and therefore development of pH (Uchide and Saida, 2005; Venco et al., 2014a; González-Miguel et al., 2015).

In heartworm disease, endarteritis and pH are closely linked. It has been postulated that the determination of pH and serological factors could be extremely useful to determine the severity of the endarteritis and thus the chronicity of the disease (Uchide and Saida, 2005; Venco et al., 2014a; Venco et al., 2014b). Nowadays, the method of choice to diagnose pH in veterinary medicine is based on transthoracic Doppler echocardiography, which pro-

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vides a noninvasive and readily available method for estimating pulmonary arterial pressure (Kellihan and Stepien, 2010). However, the diagnosis is often based on indirect and subjective parameters, specifically when tricuspid regurgitation and/or pulmonary insufficiency are difficult to obtain. Recently, the Right Pulmonary Artery Distensibility Index (RPAD Index) was validated as a valuable and objective method to estimate the presence and severity of pH in dogs infected by *D. immitis* (Venco et al., 2014b). The RPAD Index is basically calculated as the difference in diameter of the right pulmonary artery in systole and diastole as measured by M-mode.

Nevertheless no studies have been published assessing the evolution of the endarteritis and pH after the death of the parasites, so the objective of this study was to evaluate the influence that the elimination of the worms exerts over the pulmonary pressure and therefore evolution of the endarteritis, through the evaluation of the RPAD Index and other echocardiographic measurements in 2D mode, M-mode and Doppler echocardiography in dogs naturally infected by *D. immitis*.

2. Materials and methods

34 client-owned dogs brought to the Veterinary Medicine Service of the University of Las Palmas de Gran Canaria were included in the study. The dogs lived in a hyper-endemic area of *D. immitis* (Montoya-Alonso et al., 2011; Montoya-Alonso et al., 2016). Seventeen were male and 17 were female and the age ranged from 2 to 6 years; 18 were mixed-bred dogs and 16 were pure-bred dogs. 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 further evaluated for the presence or absence of microfilariae using a modified Knott test. Thoracic radiography and echocardiography exams were carried out in all dogs at the beginning and at the end of the study.

Dogs underwent echocardiographic exam by using an ultrasound machine with spectral and color Doppler and multifrequency probes (5.5–10 MHz) (Logic P5, General Electric, New York, USA). The dogs were placed in right lateral recumbence with the transducer placed in the third intercostal space. Dogs were conscious and under electrocardiographic monitoring during the whole test. For each measurement, six continuous cardiac cycles were recorded. All the echocardiographic records were carried out by the same researcher.

Echocardiographic findings were evaluated to estimate the worm burden (Venco et al., 2003), and a score from 1 to 4 was assigned from low to high worm burden as follows: (1) No worms visualized, (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, (4) worm echoes occupying the whole right pulmonary artery and the main pulmonary artery to the level of pulmonary valve.

The following parameters were determined by means of spectral Doppler: pulmonary flow acceleration time (AT), pulmonary flow ejection time (ET), AT/ET ratio, maximum velocity of the blood flow through the pulmonary artery (PAVmax) and pulmonary deceleration time (DT). Presence or absence of tricuspid regurgitation (TR) and pulmonary regurgitation (PR) as well as gradient between chambers, were also assessed. By standard two-dimensional mode, the following was determined: main pulmonary artery/aorta ratio (PA/Ao ratio), left atrial (LAV) and right atrial (RAV) volumes, left atrial (LAA) and right atrial (RAA) areas. By M-mode, the measured parameters were: fractional shortening (FS), ejection fraction (EF), right ventricular internal diameter in telediastole (RVIDD) and left ventricular internal diameter in telediastole (LVIDD), RVIDD/LVIDD ratio, right ventricle wall thickness (RVWT), left ventricle posterior wall thickness (LVPWT), RVWT/LVPWT ratio, and tricuspid annular plane systolic excursion (TAPSE). The RPAD Index was determined as described by Venco et al. (2014b).

The dogs received adulticide treatment following the American Heartworm Society recommended management protocol (American Heartworm Society, 2014). Briefly, on day 0 the dog is diagnosed and verified as heartworm positive, start with monthly heartworm preventative based on ivermectin (6 μg/kg) (Cardotek-30 Plus, Merial Laboratorios S.A., Barcelona, Spain) and the administration of doxycycline (Ronaxan, Merial Laboratorios S.A., Barcelona, Spain) (10 mg/kg BID) for 4 weeks. On day 60 the dog is treated with the first intramuscular injection of melarsomine (2.5 mg/kg) (Immiticide, Merial Laboratorios S.A., Barcelona, Spain), followed on day 90 by a second injection, and a third injection on day 91. On day 120 the dog is tested for presence of microfilariae and discharged. Finally, the day 271, 6 months after completion, an antigen test is done to confirm the adulticide efficacy.

Moderate exercise restriction was recommended since the day 0 until day 60 and significant since day 60 until at least one month after the last melarsomine injection. Echocardiographic measurements were assess on day 0 (day of the diagnosis) and day 120 (day of discharge). Measurements were assessed by the same technician who on day 120 was blinded about the results from day 0.

The data were analyzed using the SPSS Base 19.0 software for Windows. A Kolmogoroz-Smirnov test was performed to verify the normal distribution of the data. Continuous variables were expressed as median ± standard deviation. Qualitative variables are expressed as percentage. The non-parametric test of Wilcoxon was used to determine the differences before and after treatment. The Chi square 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.

All the owners gave their consent to participate in this study. The study was approved by the ethical committee of the Veterinary Medicine Service of the University of Las Palmas de Gran Canaria and was carried out in accordance with the current European legislation on animal protection.

3. Results

Based on the modified Knott test results on day 0, 17 dogs (50%) showed microfilaremia and 17 (50%) were amicrofilariemic. On day 120, only one dog showed microfilaremia.

According to the echocardiographic findings, at the beginning of the study 25 dogs (74%) were considered as having a low burden of heartworms (scores 1 or 2) while 9 (26%) were considered to have a high burden (scores 3 or 4). On day 120, all dogs were classified as score 1.

Absence or presence of pH, as well as the severity, was based on the determination of the RPAD Index. According to Venco et al. (2014b), pH was present in 68% of the dogs (n = 23): 2 dogs presented severe pH (RPAD Index ≤22%), 13 dogs presented moderate pH (RPAD Index 23–27%) and 8 presented mild pH (RPAD Index 28–35%). Normal pulmonary pressure was present in 32% (n = 11) of the studied dogs (RPAD Index ≥36%). On day 120, 23 dogs showed pH (3 dogs presented severe pH, 5 dogs presented moderate pH and 15 presented mild pH), while 11 dogs showed normal pulmonary pressure.

No significant differences were observed between the RPAD Index on day 0 and RPAD Index on day 120, in both with high and low parasite burden groups of dogs (Table 1). When the evolution of the RPAD Index was observed individually from day 0 to day 120, 6 dogs evolved from normotense to hypertense and 6 dogs evolved from hypertense to normotense; 17 dogs with pH remained hypertense on day 120, and 5 dogs which were normotense on day 0 remained normotense on day 120.

Table 1
Right Pulmonary Artery Distensibility (RPAD) Index on day 0 (beginning of the study, day of diagnosis) and day 120 (one month after the last adulticide dose) of the studied dogs (n = 34) according to parasite burden on day 0 based on echocardiographic findings (Venco et al., 2003). Dogs were considered as having a low burden of heartworms (n = 25) if no worms were visualized or few worm echoes were observed in the distal part of the right pulmonary artery, and considered to have a high burden (n = 9) if worm echoes were occupying the right pulmonary artery and extending to the main pulmonary artery or occupying the whole right pulmonary artery and the main pulmonary artery to the level of pulmonary valve.

RPAD Index (%)	High Parasite Burden (n = 9)	Low Parasite Burden (n = 25)
Day 0	31.06 ± 5.50	32.36 ± 9.01
Day 120	31.11 ± 7.09	35.33 ± 11.23

Table 2
Echocardiographic parameters measured in the studied dogs (n = 34).

Echocardiographic parameter	Day 0 (n = 34)	Day 120 (n = 34)
AT (ms)	0.08 ± 0.02	0.07 ± 0.02
ET (ms)*	0.21 ± 0.04	0.19 ± 0.02
AT/ET	0.37 ± 0.09	0.39 ± 0.10
PA*V*max (m/s)	0.84 ± 0.21	0.87 ± 0.20
DT (ms)*	0.13 ± 0.02	0.11 ± 0.02
PA/Ao	1.02 ± 0.14	0.99 ± 0.11
LAV (cm)	6.64 ± 4.98	8.13 ± 6.03
RAV (cm)	5.44 ± 3.75	5.73 ± 3.96
LAA (cm^2)	4.44 ± 1.99	5.02 ± 2.54
RAA (cm^2)	3.85 ± 1.84	3.98 ± 1.82
FS (%)	41.28 ± 10.02	37.83 ± 12.10
EF (%)	71.43 ± 12.13	67.27 ± 15.25
RVIDD (cm)	1.02 ± 0.45	0.92 ± 0.55
LVIDD (cm)*	2.98 ± 0.69	3.17 ± 0.68
RVIDD/LVIDD	0.34 ± 0.16	0.29 ± 0.19
RVWT (cm)	0.54 ± 0.12	0.55 ± 0.16
LVPWT (cm)	0.83 ± 0.25	0.76 ± 0.19
RVWT/LVPWT	0.69 ± 0.18	0.74 ± 0.20
TAPSE (cm)	1.39 ± 0.35	1.38 ± 0.32
RPAD Index (%)	32.01 ± 8.17	34.22 ± 10.37

AT: pulmonary artery flow acceleration time; ET: pulmonary artery flow ejection time; PA*Vmax*: maximum velocity of the blood flow through pulmonary artery; DT: pulmonary deceleration time; PA/Ao: main pulmonary artery/aorta ratio; LAV, RAV: left atrial and right atrial volumes; LAA, RAA: left atrial and right atrial areas; FS: fractional shortening; EF: ejection fraction; RVIDD, LVIDD: right ventricular and left ventricular internal diameters in telediastole; RVWT: right ventricular wall thickness; LVPWT: left ventricular posterior wall thickness; TAPSE: tricuspid annular plane systolic excursion; RPAD Index: Right Pulmonary Artery Distensibility Index.
* Statistically significant differences ($p < 0.05$) between day 0 and day 120.

TR and/or PR was present in 7 dogs on day 0 and 7 dogs on day 120. TR was present in 3 dogs at the beginning of the treatment with no signs of systolic pulmonary hypertension (mean velocity flow: 1.88 ± 0.69 m/s) and in 6 dogs at the end of the study (mean velocity flow: 1.56 ± 1.45 m/s). PR was present in 6 dogs at the beginning of the treatment with no signs of diastolic pulmonary hypertension (mean velocity flow: 1.22 ± 0.43 m/s), while was present in 3 dogs at the end of the study (mean: 1.81 ± 1.11 m/s).

When the other echocardiographic measurements were evaluated, only significant differences were found in DT, ET and LVIDD when measurements from day 0 and day 120 were compared ($p < 0.05$) (Table 2).

4. Discussion

Heartworm disease is characterized by arterial damage, produced by the development of proliferative endarteritis. This is characterized by the cell proliferation and migration of the vascular endothelium and smooth muscle, and the degradation of the extracellular matrix. This process results in the formation of intravascular microvilli, among other mechanisms, causing intimal thickening and narrowing of the arterial lumen (Adcock, 1961; Atwell et al., 1988; González-Miguel et al., 2012). It is caused by the presence of the adult worms in the pulmonary arteries and the lesions begin immediately upon arrival of the parasites and worsen with the chronicity of the disease (Atwell et al., 1985; Bowman and Atkins, 2009). Endarteritis leads to a decrease in pulmonary vascular compliance and an increase in pulmonary vascular resistance that, if severe, will lead to pH . When severe and chronic, pH may be complicated by right congestive heart failure (McCall et al., 2008; Bowman and Atkins, 2009).

pH is one of the most damaging complications in heartworm and is probably present in most dogs with radiographically apparent heartworm disease (Kittleson, 1998); being a frequent and severe phenomenon in heartworm, its objective determination is important. To determine the presence of pH, transthoracic Doppler echocardiography has been described as the method of choice in veterinary patients (Kellihan and Stepien, 2010). However, the diagnosis of pH was often based on indirect and subjective parameters, specifically when tricuspid regurgitation and/or pulmonary insufficiency are not present, which only help to partially quantify the disease severity. This limitation does not apply to the RPAD Index, which may be useful and predictive of pH in dogs also when tricuspid regurgitation is absent or difficult to obtain (Venco et al., 2014b; Visser et al., 2016).

Furthermore, during the adulticide treatment, the death of the worms causes thromboembolism and tends to worsen the vascular damage and presence of pH, right heart failure, or pulmonary infarction (McCall et al., 2008; Bowman and Atkins, 2009). In sight of the importance of this, there is a lack of studies evaluating the response of the pulmonary vasculature and changes in the vascular damage and pH once the parasites disappear from the system of the host.

The results from this study did not show any improvement neither worsening in the development of pH after the elimination of the parasites, independently of the parasite burden, since the differences in the RPAD Index from day 0 and day 120 were not statistically significant. In dogs with high parasite burden, previous studies showed that a significant percentage are still showing signs of pulmonary thromboembolism one month after the last injection of adulticide (Carretón et al., 2013; Carretón et al., 2014), when the second echocardiographic evaluation was carried out in this study. Thromboembolism aggravates pH, but based on the results from this study, it seems that the death of the worms did not damage significantly the vasculature of the treated dogs. This basically consists on the previous elimination of *Wolbachia* and reduction of microfilariae, followed by a stepped death of the worms, which reduces significantly the importance of the thromboembolic processes and therefore the potential exacerbation of pH in these dogs (Kramer et al., 2011). On the other hand, a study demonstrated that the biomarker of pulmonary thromboembolism D-dimer was undetectable in all dogs with low burden from the third week after the first injection until the end of the treatment, suggesting that most of the worms die after the first adulticidal injection (Carretón et al., 2013); however, neither is present any significant improvement in the RPAD Index on day 120 in dogs with low parasite burden.

These results raise the question whether more time is necessary once the intravascular parasites have been eliminated to show a significant improvement of the vasculature or if there is not any significant recovery after the adulticide treatment in dogs with chronic vascular damage. It should be considered the duration of the infection; this cannot be estimated in naturally infected dogs, but chronic infections have a significant impact in both the pathological and clinical alterations, which could influence in the capacity of recovery of the vascular system of the dog once the parasites have been eliminated. There are evidences that after the adulticidal therapy, intimal proliferation is partially reversible (Rawlings et al., 1981). Also, the C-reactive protein, an acute phase protein which probably has a role in the chronic process of remod-

eling of the arterial walls and can be used as a marker of endothelial arteritis and pulmonary hypertension in heartworm (Venco et al., 2014a), was decreased one month after the last dose of adulticide of *D. immitis*, but did not return to normal values (Méndez et al., 2015). Probably, more time is needed before appreciating some positive changes in the vasculature. It is known that the parasite antigens require a longer period of clearance from the bloodstream, and therefore circulating antigens could be still causing vascular injury (Bowman and Atkins, 2009). To investigate that, further studies should be carried out evaluating the RPAD Index in dogs several months after the treatment is finished.

On the other hand, this study showed significant differences in DT and ET. Both values decreased and this fact could be related with a more normalized flow through pulmonary artery after treatment. There were a significant different in LVIDD with a rise in this value after treatment. This fact could be related with a more normalized preload in left ventricular chamber after disappearance of worms from pulmonary arteries.

This study showed the utility and practical use of the RPAD Index in the measurement and evaluation of the hypertensive status of the dog with heartworms. Being a frequent and severe phenomenon in this disease, its objective determination is important and the possibility to establish and objective approach for the veterinary clinician to determine this status, could be beneficial for the infected dogs. The knowledge of the response and possible changes of the pulmonary vasculature after the adulticide treatment by using the RPAD Index and other echocardiographic measurements may be useful to provide an objective prognosis to the owner of the dog and also to determine if further treatment is necessary after the parasites have been eliminated.

The current results seem to indicate that there is not significant aggravation of the pulmonary damage during the death of the worms by using the adulticide protocol recommended by the American Heartworm Society, and that more time and further studies are necessary to establish the benefits of the elimination of the parasites and *Wolbachia* over the vasculature to determine the positive influence throughout the time.

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