Veterinary Parasitology 273 (2019) 1–4

Contents lists available at ScienceDirect

Veterinary Parasitology

journal homepage: www.elsevier.com/locate/vetpar

Research paper

Persistence of pulmonary endarteritis in canine heartworm infection 10 months after the eradication of adult parasites of *Dirofilaria immitis*

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ARTICLE INFO

Keywords:
Heartworm
Dirofilaria immitis
Pulmonary hypertension
Proliferative endarteritis
Echocardiographic exam
Pulmonary thromboembolism

ABSTRACT

Pulmonary endarteritis caused by *Dirofilaria immitis* and pulmonary hypertension (PH) are closely linked and the determination of PH could be validated to assess the severity and chronicity of the vascular damage, i.e. by the use of the Right Pulmonary Artery Distensibility (RPAD) Index. The aim was to evaluate the RPAD Index in dogs 10 months after the last dose of adulticide. The study included 23 client-owned dogs with heartworm brought for adulticide treatment. Echocardiographic exam was carried out to estimate worm burden, RPAD Index as well as other routine echocardiographic values on day 0 (diagnosis), day 120 (discharge), and 10 months after the last dose of adulticide. No significant differences were observed in the RPAD Index over time, neither when microfilaremic status and parasite burden were evaluated. An RPAD Index < 29% was found in 52.2% of the dogs on day 0, day 120, and 10 months after the last dose of adulticide. Regarding other echocardiographic parameters, only significant differences were observed on tricuspid annular plane systolic excursion (TAPSE) values between day 0:day 120 ($p = 0.008$), and day 0:end of the study ($p = 0.037$). There was not significant improvement in pulmonary damage 10 months after the elimination of the parasites ($p = 0.296$), suggesting that, once the endarteritis has developed, the vascular changes are chronic and may not be reversible. The modifications of the TAPSE value suggest an improvement in the systolic function of the right ventricle after the disappearance of the worms, independently of the presence of PH. The endarteritis causes a decrease in the elasticity in the pulmonary vasculature and an increase in the resistance that, chronically, and depending on the severity, will cause the development of PH and later right heart failure. This complication of the disease is one of the most damaging and frequent, so it is necessary to adequately monitor PH in dogs undergoing adulticide treatment. Furthermore, knowing the pulmonary status could provide valuable information to help provide an objective prognosis and help assess the need to add additional treatments, once the worms have been eliminated.

1. Introduction

Heartworm disease is caused by the nematode *Dirofilaria immitis,* which mainly affects canids and felids. It is a vector-borne disease whose adults lodge in pulmonary arteries and right heart chambers. During heartworm infection, damage to the endothelium occurs which, as consequence, produces intimal proliferation, which begins immediately after the arrival of the parasites (Schaub et al., 1981; Keith et al., 1983; Simón et al., 2012). This is due to the multiplication and migration of smooth muscle cells to the intima, which creates a villous proliferation in the inner surface of the artery (Atwell et al., 1985, 1988; McCall et al., 2008). As a consequence, there is a thickening of the wall of the arteries and the intimal surface presents a rough texture, causing a narrowing of the vessel lumen, especially in the small branches. As lesions develop, the arteries become stiffer and lose compliance, so there is an increase in pulmonary arterial pressure that leads to a chronic development of pulmonary hypertension (PH). Furthermore, the death of parasites triggers pulmonary thromboembolism, which also causes acute episodes of PH and contributes to the chronic development of PH (Bowman and Atkins, 2009; Simón et al., 2012).

PH is a serious condition that is frequently found in heartworm disease. It is one of the main causes that produce clinical signs in the infected dog and, if it is not treated correctly, will lead chronically to the development of right-sided cardiac disease. Moreover, there is an intimate association between pulmonary endarteritis and the presence of PH; therefore, the determination of PH can be fruitful to determine the severity and chronicity of the endarteritis (Sasaki et al., 1992; Uchide and Saida, 2005; Venco et al., 2014a).

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https://doi.org/10.1016/j.vetpar.2019.07.008
Received 27 March 2019; Received in revised form 24 July 2019; Accepted 25 July 2019

For this, the echocardiographic exam is the method of choice, which provides a simple non-invasive procedure to estimate pulmonary artery pressure in animals. In dogs with heartworm disease, previous studies have supported the usefulness of the Right Pulmonary Artery Distensibility (RPAD) Index as a useful and objective method to estimate the existence and severity of PH (Venco et al., 2014b; Visser et al., 2016), which is calculated as the difference in diameter of the right pulmonary artery in systole and diastole as measured by M-mode (Venco et al., 2014b; Serrano-Parreño et al., 2017a). This method constitutes an advantage over other echocardiographic determinations, which are based mainly on subjective and indirect parameters. The RPAD Index is especially useful when there is not tricuspid regurgitation or pulmonary regurgitation.

An earlier study showed that there was no significant worsening or recovery of damage to the pulmonary arteries in dogs with heartworm, one month after the last dose of adulticide treatment with melarsomine (Serrano-Parreño et al., 2017b), concluding that, if a significant improvement were possible after eliminating the worms and finishing the treatment, it would probably take more time before observing positive changes after the elimination of the parasites.

Therefore, the aim of this study was to evaluate the RPAD Index in dogs 10 months after the last dose of adulticide treatment, to determine changes in pulmonary pressure and, consequently, the progression of pulmonary endarteritis in these patients.

2. Materials and methods

The study recruited 23 dogs living in Gran Canaria, a hyperendemic island (Montoya-Alonso et al., 2016) diagnosed with heartworm by the detection of circulating *D. immitis* antigens (Urano test Dirofilaria®, Urano Vet SL, Barcelona, Spain) and attended the Veterinary Medicine Service of the University of Las Palmas de Gran Canaria for adulticide treatment. Microfilaremic status was assessed by a modified Knott test (Genchi et al., 2007). None of the animals received previous treatment against the disease, presented previous or concomitant pathologies and none participated in other studies.

The dogs underwent an echocardiographic exam as described previously (Serrano-Parreño et al., 2017a). Echocardiographic exams were performed on day 0 – the day of the diagnosis and when the study began –, on day 120 – one month after the last dose of melarsomine and when the dog was discharged – and 10 months after the last dose of adulticide. The worm burden was estimated by echocardiographic exam according to the guidelines established by Venco et al. (2003) and a grade from 1 to 4 was designated from low to high parasite burden.

The RPAD Index was assessed following the methodology established by Venco et al. (2014b), as well as other echocardiographic parameters were measured. By M-mode these 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). By standard two-dimensional mode, main pulmonary artery/aorta ratio (PA/Ao ratio), left atrial (LAV) and right atrial (RAV) volumes, left atrial (LAA) and right atrial (RAA) areas were determined. Finally, by means of spectral Doppler the following parameters were assessed: 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).

Adulticide treatment was administered to the recruited dogs following the current recommended management protocol (European Society of Dirofilariosis and Angiostrongylosis, 2017; American Heartworm Society, 2018), with the administration of doxycycline (10 mg/kg BID) for four weeks, monthly heartworm preventative (ivermectin: 6 µg/kg) and intramuscular administration of three doses of melasomine on days 60, 90 and 91.

On day 120, the Knott test and echocardiographic exam were performed and the dogs were discharged. Six months after the last dose of melarsomine, the efficacy of the treatment was verified with tests for the detection of antigens (Urano test Dirofilaria®, Urano Vet SL, Barcelona, Spain) and microfilariae (modified Knott test). Exercise restriction was prescribed throughout the treatment, especially from the first dose of immiticide until the dog was discharged. The monthly prophylaxis was not interrupted during the study.

The data were analysed using the SPSS Base 20.0 software for Windows. 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 between days 0, 120 and the end of the study. The Chi square test or Fisher's exact test were used to assess the association between categorical variables. In all cases, a p value < 0.05 was determined as significant.

All the owners were informed about the present study and consented to participate. The study was approved by the ethical committee of the Veterinary Medicine Service of the University of Las Palmas de Gran Canaria (MV-2015/04) and was executed in accordance with the current European legislation on animal protection.

3. Results

All the animals finished the adulticide treatment without significant complications. Nine dogs were male and 14 were female, with a range from 2 to 7 years; eight were mongrel dogs while 15 were pure-bred dogs. According to the results of the Knott test on day 0, microfilaremia was present in 11 dogs (47.8%) while 12 (52.2%) were amicrofilariemic. The day of discharge and six months after the last adulticide dose, all dogs were amicrofilariemic.

On day 0, 20 dogs (87%) showed a low parasite burden (scores 1 or 2) while three (13%) presented a high burden (scores 3 or 4) (Venco et al., 2003). On day 120 and 10 months after the last adulticide dose, no worms were echocardiographically detected in any of the dogs. Also, all dogs showed absence of circulating heartworm antigens six months after completion of the treatment.

Presence or absence of PH, as well as severity, were assessed based on the measurement of the RPAD Index. A RPAD Index $< 29\%$ [presence of PH according to Visser et al. (2016) and presence of severe-moderate PH, according to Venco et al. (2014b)] was present in 52.2% of the dogs on day 0, day 120 and 10 months after the last dose of adulticide. Fig. 1 and 2 show the evolution of RPAD Index in dogs with/ without PH based on the cut-off of Visser et al. (2016).

On day 0, mean RPAD Index was 27.4% (median: 27.22%, min: 7.9%, max: 44.6%), on day 120 it was 29.2% (median: 28.4%, min: 7.9%, max: 50%) and 10 months after the last dose of adulticide, the mean RPAD Index was 27.9% (median: 28.2%, min: 7.8%, max: 42%). No statistically significant differences were observed between the measurements of the RPAD Index when all dogs were evaluated ($p = 0.296$), neither between those dogs with RPAD Index $> 29\%$ ($p = 0.268$) or between dogs with RPAD Index $< 29\%$ on day 0 ($p = 0.174$). No statistically significant differences were found in the RPAD Index when the microfilaremic status ($p = 0.118$ on day 0; $p = 0.740$ on days 120 and 10 months) and parasite burden ($p = 1.000$ on day 0; $p = 0.698$ on day 120; $p = 0.635$ after 10 months) were evaluated.

Furthermore, when the other echocardiographic values were evaluated, only significant differences were found in the TAPSE measurements between day 0 and day 120 ($p = 0.008$), and between day 0 and 10 months after the last dose of adulticide ($p = 0.037$), when all dogs were evaluated. When the RPAD Index was assessed, only statistically significant differences were observed in the measurement of TAPSE of dogs with an RPAD Index $< 29\%$, between day 0 and day 120 ($p = 0.027$). Table 1 shows the TAPSE values observed in dogs throughout the study.

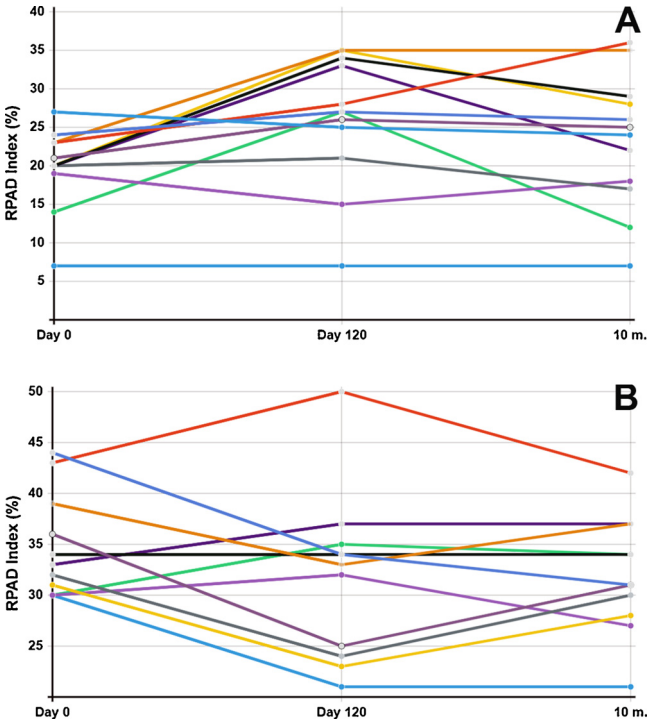

Fig. 1. Evolution of the Right Pulmonary Artery Distensibility (RPAD) Index in the studied dogs, represented by different colours. A) represents dogs with RPAD Index < 29% on day 0. This would correspond to dogs with pulmonary hypertension (PH) according to Visser et al. (2016) or dogs with severe-moderate pH according to Venco et al. (2014b). B) represents those dogs with RPAD Index > 29% on day 0, which would correspond to dogs without PH (Visser et al., 2016) or dogs with mild PH/no PH (Venco et al., 2014b). The ordinate axis of the image represents the RPAD Index (%). The axis of the abscissa shows the different measurements taken during the study (Day 0: the day of the diagnosis of the disease and beginning of the study; Day 120: one month after the last dose of melarsomine and when the dog was discharged; 10 m.: 10 months after the last dose of melarsomine).

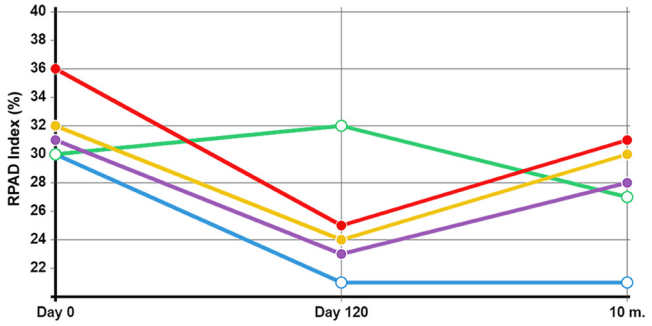

Fig. 2. Dogs with Right Pulmonary Artery Distensibility (RPAD) Index > 29% on day 0 which showed a decrease of the index below 29% at some point in the study. The ordinate axis of the image represents the RPAD Index (%). The axis of the abscissa shows the different measurements taken during the study (Day 0: the day of the diagnosis of the disease and beginning of the study; Day 120: one month after the last dose of melarsomine and when the dog was discharged; 10 m.: 10 months after the last dose of melarsomine).

No statistically significant differences were present in any of the echocardiographic parameters due to microfilaremic status or worm burden on day 0.

4. Discussion

Immediately after the arrival of the worms to the pulmonary arteries, arterial damage and development of proliferative endarteritis begin, which leads to vascular remodelling with the formation of intravascular microvilli, intimal thickening and narrowing of the lumen of the arterial vessels (Munnel et al., 1980; Schaub et al., 1981). Endarteritis worsens with the chronicity of the disease, the intensity of exercise and the immune response of the host to the parasite (Dillon et al., 1995). The influence of the parasitic load is not clear; there are studies that support its influence (Dillon et al., 1995; Méndez et al., 2015), while others state that parasite burden does not influence the development of vascular damage (Venco et al., 2014a; Serrano-Parreño et al., 2017a). In the present study, the RPAD Index was similar in dogs with high and low parasite load. However, given the low number of dogs with high load, the results should be interpreted with caution.

Table 1
Tricuspid annular plane systolic excursion (TAPSE) values on day 0 (Measurement 1: the day of the diagnosis of the disease and beginning of the study), day 120 (Measurement 2: one month after the last dose of melarsomine and when the dog is discharged) and 10 months after the last dose of melarsomine (Measurement 3). Results are expressed in cm as median, minimum and maximum values. Statistically significant differences were observed between measurement 1 and measurement 2 ($p = 0.008$) and between measurement 1 and measurement 3 ($p = 0.037$), but not between measurement 2 and measurement 3 ($p = 1.000$).

All dogs (n = 23)	Measurement 1	Measurement 2	Measurement 3
Median	1.20 cm	1.50 cm	1.40 cm
Min	0.68 cm	0.62 cm	0.81 cm
Max	1.98 cm	2.02 cm	2.17 cm

The endarteritis causes a decrease in the elasticity in the pulmonary vasculature, as well as an increase in the resistance that, chronically and depending on the severity, will cause the development of PH and the subsequent right heart failure. Also, the death of the worms causes pulmonary thromboembolisms, which worsens vascular deterioration and the presence of PH (McCall et al., 2008; Bowman and Atkins, 2009). Thus, this complication of the disease is one of the most harmful and frequent. Due to the close relationship between the presence of endarteritis and PH, it is necessary to adequately monitor this condition; for this, the RPAD Index has been useful (Venco et al., 2014b; Visser et al., 2016; Serrano-Parreño et al., 2017a).

The mechanisms of vascular damage caused by the presence of adult parasites are being widely studied (González-Miguel et al., 2015; Martini et al., 2019). However, there are no studies that assess the reaction of the pulmonary vasculature once the parasites are removed from the infected animal. So far, only one study has reported that no worsening or improvement in the evolution of PH was observed one month after the last dose of adulticide (Serrano-Parreño et al., 2017b). However, the authors reported that it may be necessary to wait longer after the adult worms, *Wolbachia* and the inflammatory response have been eliminated from the pulmonary arteries, before showing any significant improvement.

The results of the present study showed that the differences in the RPAD Index and, therefore, endarteritis, did not vary significantly 10 months after the death of the parasites. This suggests that, once endarteritis has developed, the vascular changes are chronic and may not be reversible. Furthermore, although the variations are not statistically significant, some of the dogs with RPAD Index > 29% on day 0 showed a decrease of the index below 29% at some point in the study (Fig. 3). Neither the parasite burden nor the presence/absence of microfilaremia influenced in the RPAD Index, similar to Serrano-Parreño et al. (2017b). A previous study showed evidence that, after eliminating adult parasites, endarteritis and intimal proliferation were partially reversed (Rawlings et al., 1981); however, the results of the present study suggest that the damage persists, at least 10 months after the elimination of the worms. Thus, more research should now be done to discover other indicators of endothelial damage and vascular remodelling, study other

parameters to determine the presence and severity of vascular damage, as well as to find ways to improve the dysfunction, similar to what is being done in human medicine (Mordi and Tzemos, 2014; 2016).

In a previous study, all dogs with heartworm showed anomalous TAPSE values, independently of the absence or presence of PH, similar to our results (Serrano-Parreño et al., 2017a). The current results showed that the TAPSE value presented significant differences between day 0 and day 120, as well as between day 0 and the end of the study. This may reflect an improvement in the systolic function of the right ventricle after the disappearance of the worms, despite the fact that no significant variation in pulmonary pressure was present. Likewise, it could also be due to an adaptation of the right ventricle to the chronic status of PH, as described in humans (Ghio et al., 2016). The presence of greater tricuspid insufficiency with maintained right ventricular systolic function may be another cause (Hsiao et al., 2006); however, in the present study, the presence of tricuspid insufficiency was not evaluated. These results could be beneficial for the patient since abnormal or low TAPSE values have been correlated with an increased risk of death in humans (Ghio et al., 2016).

The presence and severity of PH should be determined in dogs undergoing adulticide treatment. Knowing the pulmonary status could provide valuable information to help provide an objective prognosis and also to evaluate the need to add additional treatments to the patient, once the worms have been eliminated. For this, the RPAD Index has proven useful (Venco et al., 2014b; Serrano-Parreño et al., 2017a, 2017b).

The results obtained in this study point to the lack of significant improvements in the damage that occurs in the pulmonary arteries in dogs with heartworm, once the parasites have been eliminated using the recommended adulticide protocols, 10 months after the elimination of the worms. Additional research should be done to determine other indicators of endothelial damage and vascular remodelling.

All authors have participated in (a) conception and design, or analysis and interpretation of the data; (b) drafting the article or revising it critically for important intellectual content; and (c) approval of the final version.

Declaration of Competing Interest

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This manuscript has not been submitted to, nor is under review at, another journal or other publishing venue.

The authors have no affiliation with any organization with a direct or indirect financial interest in the subject matter discussed in the manuscript.

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