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Clinical assessment of post-adulticide complications in *Dirofilaria immitis*-naturally infected dogs treated with doxycycline and ivermectin

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ABSTRACT

This study shows that a combination of doxycycline (10 mg/kg/sid for 30 days) and ivermectin (6 μg/kg/every 15 days for 6 months) is well tolerated for the treatment of canine heartworm disease (HWD). Monthly echocardiography showed that 84% of treated dogs either progressively improved parameters indicative of pulmonary hypertension or, following slight worsening, resolved all signs. Thoracic radiography showed the persistence of interstitial inflammation, even though also in this case, approximately 70% of the dogs steadily improved or worsened but then improved by the end of the study.

1. Introduction

Heartworm infection (*Dirofilaria immitis*) in dogs causes chronic pulmonary disease that, if left untreated, can lead to right-side congestive heart failure. The presence of worms in the pulmonary arteries causes proliferation of vascular endothelium, loss of arterial elasticity and perivascular inflammation, that together cause a rise in pulmonary pressure. This can be compensated for a variable period of time, depending on worm numbers and the physical activity of the infected dog. When clinical signs occur (coughing, dyspnea, lethargy, intolerance to exercise), they are usually accompanied by altered hemodynamics on echocardiography and by varying degrees of inflammation and enlargement of pulmonary arteries and the right ventricle on thoracic radiographs (Knight, 2004). The death of adult *D. immitis*, due to either natural attrition or adulticide therapy, leads to thromboembolism as the worms are swept down into the smaller arteries, causing a worsening of cardio-pulmonary parameters (Rawlings and Calvert, 1995).

Currently, the only registered drug for adulticide therapy in dogs with heartworm disease (HWD) is melarsomine dihydrochloride (Immiticide®, Merial). Current guidelines of the American Heartworm Society (AHS) recommend a three-step treatment protocol, which allows for a more gradual elimination of the worm population. Due to concerns of severe, post-treatment thromboembolism in some dogs (Miller et al., 1995) and recent problems with availability of melarsomine on the US market, there is increasing interest in alternative adulticide treatments (Colby et al., 2011).

Long-term administration of preventive doses of ivermectin has been reported as being adulticidal in both experimentally- and naturally-infected dogs (McCall et al., 1998; Rawlings et al., 2001; Venco et al., 2004). There is concern, however, of selecting resistance to this and other macrocyclic lactones, which are the hallmark of

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disease prevention. Furthermore, Venco et al. (2004) reported that thromboembolic phenomena can be severe in some naturally infected dogs given prolonged ivermectin, even though parasites are being killed over a long period of time. The current AHS guidelines do not recommend the continuous monthly administration of prophylactic doses of any macrocyclic lactone as an adulticide protocol (www.heartwormsociety.org, 2014)

The more recent targeting of the bacterial endosymbiont *Wolbachia*, through antibiotic therapy of the infected host, has offered an interesting alternative to adulticide treatment. Indeed, Wolbachia is necessary for the reproductive capacity and long-term survival of those filarial parasites that harbor the endosymbiont, including the human filarial parasites *Onchocerca volvulus*, *Brugia malayi* and *Wuchereria bancrofti* (Bouchery et al., 2013). Several field trials using doxycycline to treat human filarial disease have reported adulticide efficacy at approximately 70% at 12–24 months after a six week regimen of daily antibiotic administration (Tamarozzi et al., 2012). The adulticide effects of doxycycline have also been studied in *D. immitis* – experimentally infected dogs by Bazzocchi et al. (2008). In this study, the authors reported no significant adulticide effects at 8 months post infection following several cycles of doxycycline, even though treatment was effective in significantly reducing *Wolbachia* populations. Interestingly, the same study reported that when doxycycline was combined with ivermectin, adulticide efficacy was approximately 80% vs. 8% when dogs were treated with doxycycline alone. This treatment regimen also lead to a notable reduction in lung pathology due to death of adult worms. More recently, Grandi et al. (2010) showed that a similar combined ivermectin/doxycycline protocol lead to clearance of infection in 75% of naturally infected dogs at 10 months after the start of treatment.

There has been no study, however, of the effect of this combined, alternative adulticide protocol on cardiopulmonary parameters used to assess thrombo-embolic events, inflammatory reactions to dying worms, and pulmonary hypertension.

The aim of the present study was to evaluate radiographic and echocardiographic findings in nineteen naturally infected dogs treated with a combination of doxycycline and ivermectin in order to assess post-adulticide complications.

2. Materials and methods

Dogs were recruited during routine clinical examination at the University of Parma Veterinary Teaching Hospital. The study protocol was approved by the University of Parma Institutional Animal Care Committee. Dogs that were positive for circulating microfilariae of *D. immitis* and/or circulating parasite antigens (according to manufacturers' instructions; PetCheck, IDEXX) and that had not received any preventive treatment in the previous 3 months were included in the study, following owners' consent. A total of 19 dogs participated in the study. Eleven were female and eight were male. Sixteen of the dogs were from the local humane shelter and it was not possible to reliably determine the exact age. However, all were adult dogs over 2 years of age. The 3 privately owned dogs ranged in age from 3 to 10 years. All dogs were treated with doxycycline (Ronaxan, Merial) at 10 mg/kg orally once daily for 30 days and with ivermectin–pyrantel pamoate (Cardotek Plus, Merial) at a minimum dose of 6 μg/kg ivermectin PO once every 15 days for 6 months. Owners were instructed to limit strenuous exercise for the duration of the study.

Dogs were submitted to complete physical examination, thoracic radiography (right lateral and dorso-ventral projections) and echocardiography at enrollment and once a month during the entire study period (days 0, 30, 60, 90, 120, 150, 180). A last complete examination was conducted 4 months after the last monthly visit (day 300 after onset of therapy).

Scoring criteria of thoracic radiographs were according to Thrall (2013) and are shown in Table 1. Thoracic radiographs were blindly assessed for interstitial lung disease and attributed a score of 0–3 by an experienced radiologist.

Scoring criteria of pulmonary hypertension by echocardiography are shown in Table 1. The scoring system was a combination of right ventricle systolic time intervals (STIs) and continuous wave (CW)-Doppler tricuspid regurgitation velocity (Schober and Baade, 2006; Kellum and Stepien, 2007). Pulsed wave (PW)-spectra signals for calculation of STIs were acquired from the right parasternal short axis view of the pulmonary artery with the sample volume at the valve level. CW-spectra of tricuspid regurgitation, when present, were acquired from the left apical cranial two-chamber views or from the right parasternal short axis view optimized to display the tricuspid valve, the

Table 1
Scoring criteria for alterations on thoracic radiography and echocardiography in dogs with natural heartworm (*Dirofilaria immitis*) infection treated with ivermectin and doxycycline.

Pulmonary interstitial disease (thoracic radiography)			
0 Normal	1 Mild unstructured interstitial lung pattern in the caudal lobe	2 More diffuse and uniform unstructured interstitial lung pattern	3 Diffuse pulmonary densities, signs compatible with thrombo-embolism/pneumonia
Pulmonary hypertension (echocardiography)			
0 Normal (AT[a] > 64 ms; AT/ET[b] ratio >0.42 ms) TRV < 2.8 m/s	1 Slight alterations (AT/ET = 0.42–0.26 ms) TRV < 2.8 m/s	2 Moderate alterations (AT ≤ 45 ms; AT/ET ≤ 0.25; TRV[c] ≥ 2.8 m/s)	3 Marked alterations (moderate/severe pulmonary hypertension: TRV > 3.57 m/s)

[a] Acceleration time.
[b] Ejection time.
[c] Tricuspide valve regurgitation velocity.