ORIGINAL ARTICLE

Efficacy and tolerability of the American Heartworm Society therapeutic protocol in dogs affected by heartworm disease without caval syndrome

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Objectives: The American Heartworm Society medical protocol represents the current standard of therapy for canine heartworm disease without caval syndrome. However, data on the tolerability of this protocol are limited. This study aimed to describe efficacy and prevalence of possible treatment-related side effects in dogs with heartworm disease treated using the American Heartworm Society protocol.

Materials and Methods: For this retrospective multi-centre cohort study, dogs diagnosed with classes 1 to 3 heartworm disease that completed the American Heartworm Society medical protocol were searched in four medical databases. Demographic, clinical, diagnostic, therapeutic and outcome data, including the number and type of possible treatment-related side effects, were retrieved.

Results: Thirty-five dogs were included. The median age and bodyweight were 6 years (1 to 13 years) and 17.3 kg (4.9 to 50 kg), respectively. Heartworm disease was classified as classes 1, 2 and 3 in 20 of 35, 11 of 35 and four of 35 dogs, respectively. In addition to the therapeutic recommendations of the American Heartworm Society, eight of 35 dogs underwent sedation to favour melarsomine administration, and 30 of 35 received ice at the injection site. After adulticide therapy, all dogs were hospitalised with cage rest [median time 12 hours (6 to 48 hours)]. All dogs survived the treatment. All dogs with long-term follow-up (32/35) became negative. Furthermore, treatment-related side effects were rare, mild and rapidly recovered without the need for supporting therapies; these included depression/lethargy (4/35 dogs), cough (2/35 dogs) and lameness, pain and gastrointestinal signs (1/35 dog each).

Clinical Significance: The American Heartworm Society medical protocol is efficient and safe in dogs with classes 1 to 3 heartworm disease.

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INTRODUCTION

Heartworm disease (HWD) is a serious infectious disease caused by the parasitic worm *Dirofilaria immitis* (Atkins, 2017; Nelson *et al.*, 2018). The organism is transmitted by several species of mosquitoes, which carry heartworm larvae [*i.e.* "microfilariae" or "L1" (first larval stage), which then mature to "L3" in the mosquito] from an infected dog to a new animal host. The larvae then grow into adult worms [*i.e.* from L3 to "L4" and then from L4 to "macrofilarie" or "L5" (fifth and last larval stage)], which preferentially live in the pulmonary arteries (Atkins, 2017; Nelson *et al.*, 2018). There, the presence of parasites can cause several complications, including pulmonary thromboembolism (PTE), pulmonary hypertension (PH), right-sided congestive heart failure (RCHF) and caval syndrome. Therefore, if not promptly diagnosed and properly treated, HWD can be fatal (Atkins, 2017; Nelson *et al.*, 2018). Although several therapeutic approaches have been investigated worldwide over time (Atkins, 2017; European Scientific Consensus Companion Animal Parasites, 2023; Nelson *et al.*, 2018), the current standard treatment of dogs without caval syndrome is represented by the medical protocol proposed by the American Heartworm Society (AHS) (https://www.heartwormsociety.org/veterinary-resources/american-heartworm-society-guidelines), as it guarantees the highest negativization rate (*i.e.* ~99%) (Atkins, 2017; Nelson *et al.*, 2018). According to this protocol, at the time of diagnosis, a macrocyclic lactone is prescribed (which should be subsequenlty readministrated every month) to prevent further infection, reduce or eliminate microfilarie and destroy developing L4 (not yet susceptible to adulticidal therapy) (Atkins, 2017; Nelson *et al.*, 2018). At the same time, doxycycline is prescribed for 4 weeks as it reduces the fertility of *D. immitis* by killing its endosymbiont (*i.e. Wolbachia pipiens*), makes macrofilarie more susceptible to the adulticide therapy, resulting in a higher kill rate, attenuates lung pathology and reduces complication and death rates in infected dogs (Atkins, 2017; Nelson *et al.*, 2017; Nelson *et al.*, 2018). Then, melarsomine dihydrochloride (an organic arsenical chemotherapeutic agent that currently represents the only Food and Drug Administration-approved product for adulticidal therapy) is introduced into the protocol 60 days after diagnosis (Atkins, 2017, Nelson *et al.*, 2018). Specifically, the AHS recommends three intramuscular administrations at specific time points (*i.e.* days 60, 90 and 91 after diagnosis) to obtain the maximal adult kill rate (~50% reduction in adult worm burden after the first injection and ~100% reduction after completion of all three doses) (Atkins, 2017, Nelson *et al.*, 2018). Furthermore, indications are provided in the protocol to prevent/reduce the chances of possible complications related to the presence/death of worms, including periods of corticosteroid administration and exercise restriction (Atkins, 2017, Nelson *et al.*, 2018).

When using a medical protocol in clinical practice, its efficacy should be considered as well as its tolerability. In this regard, it is important to underline that, although several authors have reported possible treatment-related side effects (TRSEs) (Atkins, 2017; Hettlich *et al.*, 2003; Moore *et al.*, 2013; Nelson *et al.*, 2017; Nelson *et al.*, 2018), to date, only one study has specifically focused on the prevalence and types of complications due to the use of the standard AHS protocol (Maxwell *et al.*, 2014). Moreover, it should be noted that this study reported the results from only two institutions from the southern USA. This inevitably limits our perception of the clinical safety of this medical approach and, as a consequence, our prognostic considerations in affected dogs treated according to the AHS recommendations worldwide.

Therefore, the aim of this study was to retrospectively evaluate a population of dogs with naturally occurring HWD without caval syndrome that had completed the treatment indicated by the AHS in several institutions from different geographic areas compared to the aforesaid study and to provide a detailed description of selected historical, clinical, diagnostic, therapeutic and outcome data, with emphasis not only on efficacy of AHS protocol, but also on potential TRSEs.

MATERIALS AND METHODS

Study design and inclusion criteria

For the purpose of this retrospective multi-centre cohort study, the medical records of dogs with HWD that had undergone clinical management at the authors' institutions were reviewed. A total of four institutions were involved, including three from northern Italy (*i.e.* one in Padova and two in Bologna) and one from southern Italy (*i.e.* Bari). The diagnosis of HWD was based on at least one of the following criteria: (1) positivity on both the microfilaria test (*i.e.* examination of a drop of fresh blood under a cover slip) and the antigen test for the presence of adults; and (2) negativity for the presence of microfilariae but positivity on two consecutive antigen tests from different manufacturers (Nelson *et al.*, 2018). Given the limited sensitivity of transthoracic echocardiography in the diagnosis of HWD (Nelson *et al.*, 2018), echocardiographic findings alone were not considered a sufficiently reliable diagnostic criterion for a conclusive diagnosis (*i.e.* echocardiography did not represent an alternative diagnostic tool to the aforementioned laboratory analysis, but rather a complementary investigation aimed at producing further data in dogs positive for the microfilaria test and/or antigen test). However, the echocardiographic findings were noted. Cases were included in the study if the dog had received the medical protocol described by the AHS guidelines (Nelson *et al.*, 2018) (Table 1) and completed it at our institutions within the end of the study period. Dogs were excluded from the study if they did not follow any of the indications from the aforementioned protocol (*e.g.* if they had received only one or two doses of melarsomine or if they had not received doxycycline, corticosteroids or a preventive compound), if they did not complete it within the end of the study period, if only part of the AHS medical protocol was performed at our institutions or if complete medical records were not available during the treatment period.

Table 1. Highlights of the American Heartworm Society medical protocol (Nelson *et al.*, 2018)

Day 0	Stabilise with appropriate therapy and nursing care Begin exercise restriction Prednisone (0.5 mg/kg twice a day orally 1st week, 0.5 mg/kg once a day 2nd week, 0.5 mg/kg EOD 3rd and 4th weeks)
Day 1	Heartworm preventive Observe for at least 8 hours for signs of reaction
Day 1 to 28	Doxycycline (10 mg/kg twice a day orally for 4 weeks)
Day 30	Heartworm preventive
Day 60	Heartworm preventive 1st melarsomine injection (2.5 mg/kg im) Prednisone (0.5 mg/kg twice a day orally 1st week, 0.5 mg/kg once a day 2nd week, 0.5 mg/kg EOD 3rd and 4th weeks) Decrease activity level even further (cage restriction/on leash when using yard)
Day 90	Heartworm preventive 2nd melarsomine injection (2.5 mg/kg im) Prednisone (0.5 mg/kg twice a day orally 1st week, 0.5 mg/kg once a day 2nd week, 0.5 mg/kg EOD 3rd and 4th weeks)
Day 91	3rd melarsomine injection (2.5 mg/kg im) Continue exercise restriction for 6 to 8 weeks
Day 120	Test for presence of microfilariae
Day 365	Antigen test

EOD Every other day, im Intramuscularly
All enrolled dogs had to follow all indications of this protocol

As this was a retrospective investigation performed on non-experimental dogs that exclusively underwent selected diagnostic and therapeutic procedures made necessary by their underlying heart disease (*e.g.* HWD), no additional stress was imposed on the animals to obtain the data used in this study. Therefore, no institutional animal use approval was sought.

Medical record search

The following medical record search strategy of dogs presented in the authors' hospitals between January 2014 and May 2023 was employed by four operators (one for each hospital included in the study): using the hospital management softwares (Fenice 5.49, ZakSoft Srl, Bologna, Italy; EasyVet, Snoots S.r.l., Milano, Italy) integrated medical records search function, the list of all dogs that were presented to the hospitals was evaluated. The medical records system was then searched based on the following keywords: "Dog," "HWD" and "*D. immits*." Using these criteria, an electronic spreadsheet (Microsoft Excel version 2016, Microsoft Corporation, Redmond, USA) was created that included one row for each individual consultation.

Data extracted

For dogs that met the inclusion criteria, additional information was acquired from medical records, including signalment, history, physical examination, diagnostic tests performed before the start of treatment and the pretreatment classification of the HWD. Regarding signalment, dogs were classified according to their bodyweight as small (< 9 kg), medium (from 9 to <30 kg), large (from 30 kg to <40 kg) and giant (> 40 kg) (Wallis *et al.*, 2021). Information on diagnostic tests included the number and type of abnormalities in the complete blood count, serum chemistry, urinalysis, thoracic radiography and transthoracic echocardiography. With specific regard to echocardiographic analysis, the assessment of PH was based on characteristic cardiac changes occurring secondary to PH (*e.g.* interventricular septum flattening, right ventricular hypertrophy and/or enlargement of the pulmonary artery, right atrium and/or caudal vena cava) and by estimating pulmonary arterial pressure from spectral Doppler tracings [*i.e.* continuous-wave Doppler interrogation of tricuspid regurgitation and/or pulmonary insufficiency jets, using a simplified Bernoulli equation (pressure gradient = $4\times v^2$)], following the current consensus statement guidelines of the American College of Veterinary Internal Medicine (Reinero *et al.*, 2020). Based on clinical signs and diagnostic abnormalities, the dogs were classified into different classes of severity of HWD, adapting the previously described classification system (Maxwell *et al.*, 2014). Specifically, Class 1 dogs were positive animals without any or with only mild clinical signs (*e.g.* occasional cough) and showing no abnormalities on thoracic radiographs and laboratory tests. Class 2 dogs had mild-to-moderate clinical signs (*e.g.* exercise intolerance) and mild-to-moderate abnormalities on thoracic radiographs (*e.g.* pulmonary artery enlargement) and laboratory tests (*e.g.* anaemia with a packed cell volume of 20 to 30%). Class 3 dogs had moderate-to-severe clinical signs (*e.g.* persistent cough, dyspnoea, abdominal distension due to RCHF) and moderate-to-severe abnormalities on thoracic radiographs (*e.g.* right-sided cardiac enlargement) and laboratory tests (*e.g.* anaemia with a packed cell volume < 20%) but without evidence of caval syndrome. Class 4 dogs had caval syndrome. This was defined as an acute syndrome characterised by a heavy worm load, in which the worm mass is present in the right cardiac chambers and across the tricuspid valve, inducing fragmentation of red blood cells with consequent severe regenerative anaemia and haemoglobinuria due to intravascular haemolysis (Atkins, 2017; Maxwell *et al.*, 2014; Nelson *et al.*, 2018). For the purpose of this study, only dogs of classes 1 to 3 were enrolled, whereas dogs in class 4 were excluded.

Treatment(s) administered

Information on treatment was recorded, including the type of preventive heartworm, the administration of adjunctive therapies other than those recommended by the AHS (*e.g.* sildenafil in case of severe PH or furosemide for dogs with RCHF), the possible need for sedation to favour intramuscular administration of melarsomine, the use of ice to reduce pain/swelling at the injection site and the duration of possible hospitalisations to favour cage rest. The type and duration of possible TRSEs throughout the treatment period as well as the need for additional supporting therapies prescribed for these complications were retrieved from the medical record. The last dose of melarosimine is administered conventionally 91 days after HWD diagnosis and adverse signs have been reported up to 8 weeks after the administration of this drug (Atkins, 2017; Moore *et al.*, 2013; Nelson *et al.*, 2018), the treatment period during which possible TRSEs were monitored included the first 5 months after HWD diagnosis. Lastly, medical records and referral veterinarians were also screened for information regarding follow-up HWD testing and long-term outcomes. For the purpose of this study, the presence of concurrent diseases

or ongoing therapies not related to HWD was not considered an exclusion criterion, although its presence was noted.

Statistical methods

Descriptive statistics were performed. After testing for normality using the Shapiro–Wilk test and visual inspection, normally or non-normally distributed continuous variables were reported as mean and standard deviation and median (minimum-maximum range), respectively.

RESULTS

Patient inclusion, signalment and anamnesis

In total, 87 dogs were diagnosed with HWD infection during the study period. However, 52 dogs were not included in the study as they did not follow some indication of the AHS medical protocol (31 dogs), only part of the AHS medical protocol was performed at our institutions (16), they did not complete the AHS protocol till the end of the study (three dogs) or they had a class 4 HWD (two dogs). Therefore, 35 dogs met the inclusion criteria (13 from Padova, 19 from Bologna and three from Bari). The demographic and clinical characteristics of the study population are reported in Table 2. Based on bodyweight, 10 of 35 (28.6%) dogs were classified as small, 19 of 35 (54.3%) dogs as medium, one of 35 (2.86%) dogs as large and five of 35 (14.3%) dogs as giant.

Table 2. Selected demographic and clinical data of dogs with heartworm disease enrolled in this study

Variable	
No. of enrolled dogs with HWD treated according to the AHS medical protocol	35
Age (years)	6 (1 to 13)
Bodyweight (kg)	17.3 (4.9 to 50)
Sex (EM/NM/EF/NF)	13/5/11/6
Breed (No. of dogs)	Mixed breed (18) Maremma Sheepdog and English Setter (3 each) Alaskan malamute, American Staffordshire terrier, Dogo, English Bulldog, French Bulldog, German shepherd dog, Irish Wolfhound, Podenco, Pug, Rottweiler and Yorkshire terrier (1 each)
Concurrent diseases other than HWD (Y/N)	14/19
Type of concurrent diseases (N. of dogs)	Stage B2 MMVD, cruciate ligament rupture and inflammatory gastroenteritis (2 each) Stage B1 MMVD, CKD, Cushing's disease, dermatitis, epileptic seizures, gastric dilation, intervertebral disc herniation and mammary nodule (1 each)
Concurrent therapies other than those for HWD (Y/N)	7/24
Type of concurrent therapies (No. of dogs)	Antibiotics, omeprazole and pimobendan (2 each) Diazepam (1)

AHS American Heartworm Society, CKD Chronic kidney disease, EF Entire female, EM Entire male, HWD Heartworm disease, MMVD Myxomatous mitral valve disease, NF Neutered female, NM Neutered male

Physical examination, clinicopathologic and diagnostic imaging findings

Clinical signs related to HWD were presented in 15 of 35 (42.9%) dogs, including exercise intolerance and cough (four dogs each), dyspnoea (three dogs) and abdominal distension and syncope (two dogs each). Abnormalities documented in the complete blood count and serum chemistry at the time of diagnosis of HWD are reported in Table 3, whereas those identified on thoracic radiography and echocardiography are reported in Table 4. In addition to the diagnostic findings reported in these tables, five of 35 (14.3%) dogs had proteinuria on urinalysis; the median urinary protein-to-creatinine ratio was 1.3 (0.6 to 2.1).

Diagnosis and clinical management

According to clinical and diagnostic findings, dogs were diagnosed as HWD affected in classes 1, 2 and 3 in 20 of 35 (57.2%), 11 of 35 (31.4%) and four of 35 (11.4%) cases, respectively.

At the time of the initiation of the AHS medical protocol, some dogs received additional therapies aimed at treating concurrent complications (*i.e.* severe PH and RCHF); these included sildenafil and furosemide (in two dogs each) and benazepril, enalapril and spironolactone (in one dog each). From day 0 of the HWD diagnosis, all dogs followed the AHS recommendations, including exercise limitation (*i.e.* home restriction with the possibility of moving outside only on leash and exclusively for

Table 3. Laboratory abnormalities documented on complete cell count and serum chemistry at initial presentation in 35 dogs with heartworm disease

Laboratory finding	Number of dogs (%)
Complete blood count	
Eosinophilia	12/35 (34.3)
Neutrophilic leukocytosis	8/35 (22.9)
Non-regenerative anaemia	7/35 (20)
Serum chemistry	
Increased CRP	9/35 (25.7)
Decreased albumin	7/35 (20)
Increased ALT	5/35 (14.3)
Increased AST	3/35 (8.6)
Increased CK	3/35 (8.6)
Increased creatinine	3/35 (8.6)

ALT Alanine aminotransferase, AST Aspartate aminotransferase, CK Creatine kinase, CRP C-reactive protein

Table 4. Thoracic radiographic and echocardiographic abnormalities documented at initial presentation in 35 dogs with heartworm disease

Imaging finding	Number of dogs (%)
Thoracic radiography	
Interstitial/bronchointerstitial pattern	6/35 (17.1)
Right-sided cardiomegaly	5/35 (14.3)
Dilated/tortuous pulmonary artery(ies)	5/35 (14.3)
Echocardiography	
Evidence of heartworms	16/35 (45.7)
Doppler-PH	6/35 (17.1)
Right-sided cardiac dilatation	5/35 (14.3)
Right ventricular wall thickening	5/35 (14.3)
IVS paradoxical movement	2/35 (5.7)

Doppler-PH Diagnosis of pulmonary hypertension based on continuous-wave Doppler interrogations of tricuspid and/or pulmonary insufficiency jets, IVS Interventricular septum

urination and defecation) for 4 weeks after the first melarsomine injection and at least 6 weeks after the last melarsomine administration. Regarding heartworm preventive, four of 35 (11.4%) and 31 of 35 (88.6%) dogs received topical administration of selamectin and oral administration of ivermectin, respectively. The only additions to the AHS protocol included the use of sedatives to favour intramuscular administration of melarsomine in uncooperative dogs, the use of ice at the site of injection of melarsomine to reduce local side effects and hospitalisation to favour cage rest after adulticide therapy. Sedation was used in eight of 35 dogs (22.9%) according to different sedative protocols, including butorphanol and acepromazine (five dogs), butorphanol, acepromazine and alfaxalone (one dog), butorphanol and propofol (one dog) and butorphanol, medetomidine and propofol (one dog). Sedation was uneventful in all cases. Ice was applied at the injection site for ≤15 minutes in 30 of 35 (85.7%) dogs. Regarding hospitalisation after melarsomine administration, this occurred at the time of the first injection (*i.e.* day 61) and at the time of the second and third injections (*i.e.* days 90 to 91) in all dogs. The median hospitalisation duration was 12 hours (6 to 48 hours).

TRSEs were observed in eight of 35 (22.9%) dogs. They were usually mild and recovered in a short period of time (*i.e.* ≤48 hours), without the need for supporting therapies in all cases. Complications included depression/lethargy [4/35 (11.4%) dogs; it occurred within 24 hours after the first injection of melarsomine in three dogs and within 24 hours after the second injection of melarsomine in the remaining case], cough [2/35 (5.7%) dogs; it occurred 2 days after the first injection of melarsomine in one case and within 24 hours after the second injection of melarsomine in the remaining case], vomiting and diarrhoea [1/35 (2.9%) dog; they occurred within 24 hours after the first injection of melarsomine], lameness [1/35 (2.9%) dog; it occurred within 24 hours after the second injection of melarsomine] and pain at the injection site [1/35 (2.9%) dog; it occurred within 24 hours after the first injection of melarsomine]. The TRSEs were documented in five small dogs, two medium dogs and one large dog.

Outcomes

All dogs survived the AHS medical protocol. Long-term outcome data were available for 32 of 35 (91.4%) dogs, as the follow-up was lost in three dogs from 150 to 160 days after beginning HWD treatment. All dogs with a complete long-term follow-up became negative according to the combination of the microfilaria test performed on day 120 and the antigen test for adult presence performed on day 365. Furthermore, 22 of 35 (62.9%) dogs underwent an echocardiographic and radiographic recheck within the latter time point, showing no residual imaging abnormalities referring to HWD (*i.e.* the radiographic and echocardiographic findings had normalised in all dogs).

DISCUSSION

This study focused on the outcome of dogs with naturally occurring HWD in classes 1 to 3 treated according to the AHS recommendations. Our results demonstrated that the AHS medical protocol is not only effective but also well tolerated in these dogs.

Regarding the efficacy of the AHS protocol, our results further confirm findings from previous publications (Atkins, 2017; Nelson *et al.*, 2018; Vezzoni *et al.*, 1992), as we obtained a 100% negativisation by combining the results of the test for the presence of microfilariae and the antigen test for the presence of adults. Furthermore, dogs with an echocardiographic and radiographic recheck showed normalisation of imaging findings (*e.g.* resolution of PH, reverse remodelling of the right cardiac chambers and disappearance of heartworms on echocardiography) at the end of treatment. In other words, we demonstrated the success of HWD treatment within 1 year after diagnosis in all dogs with long-term follow-up from four different institutions. With specific regard to imaging findings, it is important to underline that the lack of residual abnormalities referring to HWD after the completion of the AHS protocol should be strictly interpreted in the light of specific features of our study population (in other words, this result should not be systematically extended to each dog that could be managed in clinical practice). Indeed, it has been demonstrated that the cardiac and lung lesions depend on both the time and magnitude of infection; therefore, in some dogs, it could be possible to observe the persistence of some imaging changes (*e.g.* interstitial signs or pulmonary artery distension on thoracic radiographs) despite treatments (Rawlings, 1986a, 1986b).

Although this study was not designed to directly compare the efficacy of different treatments (as we did not include control groups treated with medical protocols different from the one proposed by the AHS), the 100% negativisation rate documented herein could partially contribute to understand why many authors consider the AHS medical strategy preferable to other alternative protocols proposed over time, including the protocol with only two doses of melarsomine and the off-label "slow-kill" protocol (*i.e.* a protocol based on the long-term use of macrocyclic lactones instead of melarsomine to achieve adult death) (Atkins, 2017, Nelson *et al.*, 2018). Indeed, when only two doses of melarsomine are administered, only 90% of adult worms can be killed and only 70% of dogs become negative (Keister *et al.*, 1992). Similarly, with the "slow-kill" protocol, a variably limited (*i.e.* ~from 18 to ~95%) efficacy (*i.e.* defined as the percentage of worms absent at necroscopy in experimental infections and as the percentage of negative at the serological test for naturally acquired infections) can be obtained (Jacobson & DiGangi, 2021), depending on the type of macrocyclic lactone used and the duration of administration [*e.g.* adulticidal efficacy of ~95% after 10 months of moxidectin combined with doxycycline (Savadelis *et al.*, 2017), ~95% after 31 months of ivermectin (McCall *et al.*, 2001) and ~40% after 18 months of selamectin (Dzimianski *et al.*, 2001)]. According to experts' opinion, the lack of 100% negativisation rate represents a drawback, as with rates lower than 100% a true cure for HWD is not achieved; therefore, infected dogs treated with alternative protocols likely continue to be damaged by the parasites (Moorhead, 2018). Moreover, the indiscriminate use of the "slow-kill" protocol has probably contributed to the development of the ongoing epidemiologic plague named macrocyclic lactone resistance (*i.e.* the

development of heartworms resistant to these molecules) (Bowman, 2012). All these reasons explain why, in our institutions, we preferentially treat dogs with HWD according to the AHS recommendations.

Additional clinically useful findings of this study are those related to the tolerability of the AHS protocol. We decided to focus on the study of possible TRSEs, mainly because one of the sources of concern related to the AHS recommendations is the risk of possible complications (especially those related to melarsomine) (Ku, 2017). In our opinion, the perception of possible TRSEs has been biased over time. Before 2014, this bias was due to the lack of studies specifically designed to systematically and rigorously address the rate of complications related to the standard AHS protocol. After that year, perception was biased by the fact that the only study published on this topic reported a high prevalence of TRSEs, including severe ones (Maxwell *et al.*, 2014). Specifically, in the study by Maxwell et al., only 36% of 50 dogs were free of adverse effects during the medical protocol, with ~50% of the dogs experiencing minor complications (*e.g.* injection site reactions, gastrointestinal signs and lethargy/depression), ~50% of the dogs experiencing respiratory signs (*e.g.* coughing and dyspnoea potentially related to PTE) and 6% of the dogs developing heart failure during/after heartworm treatment; in addition, 14% of the dogs died within the treatment period (Maxwell *et al.*, 2014). These findings are different from those observed in our study population, as possible TRSEs documented herein were infrequent, mild as well as rapidly and spontaneously recovering in all dogs; moreover, all dogs survived treatment.

Although the reasons for this discrepancy are not immediately clear, several factors may have contributed, at least in part, to the low complication rate in our dogs. First, some dogs received a sedative [preferentially using the anaesthetic drugs suggested for dogs with HWD (Quandt, 2023)] prior to melarsomine administration to minimise patient discomfort and facilitate proper injection into the belly of the epaxial muscle. In our experience, this is particularly important in uncooperative dogs as, without sedatives, there is the risk of injecting melarsomine while the dog is moving. This, in turn, may increase the probability that the compound migrates out of the injection site through the fascial planes and causes ascending inflammation along the nerve roots, with consequent compression of the extradural cord secondary to inflammation and necrosis of epidural fat. Alternatively, an inappropriate injection technique can result in the direct contact of melarsomine with neural tissue. In both cases, a variety of neurologic complications can develop, both reversible and non-reversible, including ataxia and paraparesis (Hettlich *et al.*, 2003; Moore *et al.*, 2013). Therefore, it could be hypothesised that sedation could have played a possible role in the prevention of the complication associated with melarsomine administration, at least in uncooperative dogs. Second, ice was maintained at the injection site in approximately 85% of the dogs to minimise injection site reactions after melarsomine administration. Potentially, this may have contributed to limit immediate local inflammation and pain (El-Deen & Youssef, 2018; Travell, 1955). Third, all dogs from this study were hospitalised after melarsomine administrations. This allowed us not only to closely monitor and promptly treat possible acute side effects but also to obtain a rigorous cage rest during the first hours/days after injection. Limiting physical activity is an essential part of treatment and, according to experts' opinion, the more rigorous its execution is, the lower the rate of possible complications (Atkins, 2017; Nelson *et al.*, 2018). In fact, once the worms die as a result of adulticidal therapy, they collapse and are forced by blood flow to the distal branches of the pulmonary arteries. There, dead worms not only create a mechanical obstruction for blood flow but also cause inflammation and platelet aggregation, increasing the risk of PTE. During periods of increased activity, increased blood flow to the obstructed vessels can cause further vascular inflammation and pressure increase, favouring the chances of PTE and RCHF (Case *et al.*, 1995; Hoskins *et al.*, 1985; Rawlings *et al.*, 1993). The interplay between physical activity and the rate of possible complications is further underlined by an experimental study demonstrating that dogs infected with a higher worm burden via surgical transplantation (*i.e.* 50 heartworms) and exercise-restricted took longer to develop clinical disease and developed less pulmonary vascular disease than dogs with a lower worm burden (*i.e.* 14 heartworms) and allowed moderate activity (Dillon *et al.*, 1995). This may help explain why the rigorous exercise restriction achieved immediately after melarsomine injection could have partially contributed in reducing the chances of possible immediate complications. Moreover, owners were instructed to perform a rigorous exercise restriction after discharge for the following weeks (*i.e.* four weeks after the first melarsomine injection and at least six weeks after the last melarsomine administration) to further reduce the chance of TRSEs, as recommended by the AHS (Nelson *et al.*, 2018). However, the possible beneficial role of our additions to the AHS protocol (*i.e.* sedation, local cooling and hospitalisation with cage rest) should be interpreted as simple hypotheses, since a control group (*i.e.* a group of dogs not receiving the aforementioned additional therapeutic strategies) was lacking in this study. Therefore, further prospective investigations enrolling both dogs treated with the "standard" AHS medical protocol and dogs treated with the AHS medical protocol associated with sedation, local cooling and hospitalisation are needed to confirm or refute our hypotheses.

An additional possible explanation for the discrepancy in terms of rate of TRSEs between the present study and the one from Maxwell *et al.* (2014) may concern the use of corticosteroids. In fact, all dogs in this study received this type of drug [according to the AHS medical protocol (Nelson *et al.*, 2018)], while Maxwell et al. prescribed corticosteroids only to some of the enrolled dogs (*i.e.* ≤62%) (Maxwell *et al.*, 2014). The advantages of corticosteroid administration in dogs with HWD include the control of clinical signs of PTE, the reduction of pulmonary arteritis and the minimisation of potential adverse reactions associated with the death of parasites after the administration of macrocyclic lactones and melarsomine (Atkins, 2017; Nelson *et al.*, 2018).

At first glance, a last hypothesis for the low complication rate documented here may be due to the low prevalence of dogs with a high class of HWD. In fact, most of the dogs in this study were asymptomatic (*i.e.* 57.2% of the dogs had class 1 HWD).

However, it should be noted that, also in the study of Maxwell et al., dogs with class 1 HWD were overrepresented and that, despite such a prevalence, the complication rate was high in that study population (Maxwell *et al.*, 2014). Therefore, it seems reasonable to hypothesise that other factors, such as the type of medical treatment and/or the host's immune reaction to the clearance of dead worms, rather than the sole class of HWD, played the main role in determining the different rates of complication in the two study populations. Regarding the class of dogs enrolled, it is also important to note that, in this study, no dog had class 4 HWD (*i.e.* HDW associated with caval syndrome), as this stage represented an exclusion criterion. This choice was based on the fact that the recommended first-line treatment for dogs with class 4 HWD is represented by a therapeutic option different from the AHS medical protocol, namely minimally invasive heartworm removal (Arita *et al.*, 2003; Bové *et al.*, 2010; Cavaliere *et al.*, 2017). Therefore, the results of this study should be applied only to dogs with classes 1 to 3 HWD.

Another interesting finding from the present report concerns the signalment of enrolled dogs and, more specifically, their bodyweight. In fact, some veterinarians tend to discourage owners of large/giant-breed dogs from using the AHS protocol as they hypothesise a higher predisposition to complications in larger dogs, especially those related to melarsomine injections. According to such a hypothesis, this could be due to the fact that large/giant-breed dogs inevitably need a larger injection of melarsomine, with an increased risk of deposition of the drug in the fascial planes and/or leakage into the subcutaneous tissues. According to this theory, the recommendation is to limit the injection volumes to <4 mL/injection site (Hettlich *et al.*, 2003; Moore *et al.*, 2013). In this regard, it is important to note that we did not document a higher rate of complications among large/giant-breed dogs in our study population. In fact, we observed possible TRSEs in seven dogs weighing <30 kg and only one dog weighing ≥30 kg. Therefore, our results tend to confute the aforementioned theory and further underline the good clinical tolerability of the AHS protocol in dogs, including large or giant breeds.

This study should be read in the context of some limitations. First, the retrospective design of our analysis precluded treatment standardisation (*e.g.* not all dogs received ice at the injection site after melarsomine administration). Second, the number of dogs enrolled was relatively limited. This may be due to the fact that, according to our experience, in our country, HWD cases are not as common as in the past, most likely due to the more rigorous prescription and administration of heartworm preventive medications. Indeed, contrary to the past decades, when heartworm preventives were administered only for some months of the year (*e.g.* during spring and summer), in recent years, numerous veterinarians have begun to prescribe heartworm prophylaxis year-round (*e.g.* administration of macrocyclic lactones every month of the year) since *D. immitis* is highly diffuse/endemic in many regions of our country (Mendoza-Roldan *et al.*, 2020; Nelson *et al.*, 2018; Panarese *et al.*, 2022). Moreover, it should be noted that the results of this study only apply to dogs that completely adhere to all indications of the AHS medical protocol (*e.g.* they do not apply to dogs not receiving corticosteroids). Lastly, in this study, we analysed only routine blood tests (*i.e.* complete blood count and serum biochemistry), while cardiopulmonary markers (*e.g.* cardiac troponin I) and inflammatory markers (*e.g.* inflammatory cytokines) were not systematically investigated. Close monitoring of these laboratory parameters before, during and after melarsomine administration could have provided further information on the response to the medical protocol (Yoon *et al.*, 2017).

In conclusion, our study demonstrates that, in dogs with HWD classes 1 to 3, the AHS medical protocol is not only efficient but also safe if properly performed, especially when associated with additional precautions, namely sedation for melarsomine administration in uncooperative dogs, ice application at the injection site and hospitalisation with cage rest after adulticide therapy.

Conflict of interest

None of the authors of this article has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

Author contributions

Giovanni Romito: Conceptualization (lead); data curation (lead); formal analysis (lead); investigation (lead); methodology (equal); supervision (lead); writing – original draft (lead); writing – review and editing (equal). **Elisa Pane:** Data curation (supporting); formal analysis (supporting); investigation (supporting); methodology (supporting); project administration (supporting). **Carlo Guglielmini:** Data curation (supporting); formal analysis (supporting); project administration (supporting); resources (supporting); supervision (supporting); writing – original draft (supporting); writing – review and editing (supporting). **Helen Poser:** Data curation (supporting); investigation (supporting); methodology (supporting); writing – original draft (supporting); writing – review and editing (supporting). **Carlotta Valente:** Data curation (supporting); investigation (supporting); methodology (supporting); writing – original draft (supporting); writing – review and editing (supporting). **Paola Paradies:** Data curation (supporting); investigation (supporting); methodology (supporting); writing – original draft (supporting); writing – review and editing (supporting). **Prisca Castagna:** Data curation (supporting); investigation (supporting); methodology (supporting). **Chiara Mazzoldi:** Data curation (supporting); investigation (supporting); methodology (supporting). **Mario Cipone:** Resources (supporting); writing – original draft (supporting); writing – review and editing (supporting).

Data availability statement

The dataset analysed during the current study are not publicly available due to the potential to compromise participant consent or confidentiality but may be available from the corresponding author upon reasonable request.

References

Arita, N., Yamane, I. & Takemura, N. (2003) Comparison of canine heartworm removal rates using flexible alligator forceps guided by transesophageal echocardiography and fluoroscopy. *Journal of Veterinary Medical Science*, **65**, 259–261.

Atkins, C. (2017) Canine and feline heartworm disease. In: Ettinger, S.J., Feldman, E.C. & Côté, E. (Eds.) Textbook of veterinary internal medicine, 8th edition. St. Louis, MO: Elsevier, pp. 1316–1344.
Bové, C.M., Gordon, S.G., Saunders, A.B., Miller, M.W., Roland, R.M., Achen, S.E. et al. (2010) Outcome of minimally invasive surgical treatment of heartworm caval syndrome in dogs: 42 cases (1999-2007). *Journal of the American Veterinary Medical Association*, **236**, 187–192.
Bowman, D.D. (2012) Heartworms, macrocyclic lactones, and the specter of resistance to prevention in the United States. *Parasites & Vectors*, **5**, 138.
Case, J.L., Tanner, P.A., Keister, D.M., Meo, N., Soll, M. & Knight, D. (1995) A clinical field trial of melarsomine dihydrochloride (RM340) in dogs with severe (class 3) heartworm disease. In: Proceedings of the 1995 Heartworm Symposium. Auburn, AL: American Heartworm Society, pp. 243–250.
Cavaliere, L., Romito, G., Domenech, O. & Venco, L. (2017) Heartworm removal guided by transesophageal echocardiography in a dog with naturally acquired caval syndrome. *Journal of the American Animal Hospital Association*, **53**, 96–100.
Dillon, A.R., Brawner, W.R. & Hanrahan, L. (1995) Influence of number of parasites and exercise on the severity of heartworm disease in dogs. In: Proceedings of the 1995 Heartworm Symposium. Auburn, AL: American Heartworm Society, p. 113.
Dzimianski, M.T., Mccall, J.W., Steffens, W.L. et al. (2001) The safety of selamectin in heartworm infected dogs and its effect on adult worms and microfilariae. In: Seward, L. (Ed.) Proceedings of the 2001 American Heartworm Symposium. Batavia, IL: American Heartworm Society, pp. 135–140.
El-Deen, D.S. & Youssef, N.F.A. (2018) The effect of cryotherapy application before versus after subcutaneous anticoagulant injection on pain intensity and hematoma formation: a quasi-experimental design. *International Journal of Nursing Sciences*, **5**, 223–229.
European Scientific Consensus Companion Animal Parasites. (2023) *Control of vector-borne diseases in dogs and cats*. Available at: https://www.esccap.org/uploads/docs/5y4xn3fr_0775_ESCCAP_Guideline_GL5_20221228_1p.pdf [Accessed 5th May 2023]
Hettlich, B.F., Ryan, K., Bergman, R.L., Marks, S.L., Lewis, B.C., Bahr, A. et al. (2003) Neurologic complications after melarsomine dihydrochloride treatment for *Dirofilaria immitis* in three dogs. *Journal of the American Veterinary Medical Association*, **223**, 1456–1461.
Hoskins, J.D., Hribernik, T.N. & Kearney, M.T. (1985) Complications following thiacetarsamide sodium therapy in Louisiana dogs with naturally-occurring heartworm disease. *The Cornell Veterinarian*, **75**, 531–539.
Jacobson, L.S. & DiGangi, B.A. (2021) An accessible alternative to melarsomine: "Moxi-Doxy" for treatment of adult heartworm infection in dogs. *Frontiers in Veterinary Science*, **8**, 702018.
Keister, D.M., Dzimianski, M.T., McTier, T.L., McCall, J.W. & Bown, J. (1992) Dose selection and confirmation of RM 340, a new filaricide for the treatment of dogs with immature and mature *Dirofilaria immitis*. In: Proceedings of the 1992 American Heartworm Synposium. Batavia, IL: American Heartworm Society, pp. 241–247.
Ku, T.N. (2017) Investigating management choices for canine heartworm disease in northern Mississippi. *Parasites & Vectors*, **10**, 474.
Maxwell, E., Ryan, K., Reynolds, C. & Pariaut, R. (2014) Outcome of a heartworm treatment protocol in dogs presenting to Louisiana State University from 2008 to 2011: 50 cases. *Veterinary Parasitology*, **206**, 71–77.
McCall, J.W., Roberts, R.E., Suakornjej, M.S. et al. (2001) Further evidence of clinical prophylactic (reach-back) and adulticidal activity of monthly administration of ivermectin and pyrantel pamoate in dogs experimentally infected with heartworms. In: Seward, L. (Ed.) Proceeedings of the 2001 American Heartworm Symposium. Batavia, IL: American Heartworm Society, pp. 189–200.
Mendoza-Roldan, J., Benelli, G., Panarese, R., Iatta, R., Furlanello, T., Beugnet, F. et al. (2020) Leishmania infantum and *Dirofilaria immitis* infections in Italy, 2009-2019: changing distribution patterns. *Parasites & Vectors*, **13**, 193.
Moore, S.A., Mariani, C.L., Van Wettere, A. & Borst, L.B. (2013) Chronic compressive myelopathy and progressive neurologic signs associated with melarsomine dihydrochloride administration in a dog. *Journal of the American Animal Hospital Association*, **49**, 389–393.
Moorhead, A. (2018) *The AHS Protocol vs. "Slow Kill"*. Available at: https://www.heartwormsociety.org/veterinary-resources/veterinary-education/ahs-board-speaks-out/507-the-ahs-protocol-vs-slow-kill [Accessed 10th May 2023]
Nelson, C.T., Myrick, E.S. & Nelson, T.A. (2017) Clinical benefits of incorporating doxycycline into a canine heartworm treatment protocol. *Parasites & Vectors*, **10**, 515.
Nelson, C.T., McCall, J.W., Jones, S. et al. (2018) Current canine guidelines for the prevention, diagnosis, and management of heartworm (*Dirofilaria immitis*) infection in dogs (revised 2018). American Heartworm Society. Available at: https://www.heartwormsociety.org/veterinary-resources/american-heartworm-society-guidelines [Accessed 10th May 2023]
Panarese, R., Iatta, R., Beugnet, F. & Otranto, D. (2022) Incidence of *Dirofilaria immitis* and *Leishmania infantum* infections in sheltered dogs from Southern Italy. *Transboundary and Emerging Diseases*, **69**, 891–894.
Quandt, J. (2023) Anesthesia for the dog with heartworm disease: a brief, practical review. *Parasites & Vectors*, **16**, 151.
Rawlings, C.A. (1986a) The pulmonary arterial response to adults *Dirofilaria immitis*. In: Rawlings, C.A. (Ed.) Heartworm disease in dogs and cats. Philadelphia, PA: W.B. Saunders, pp. 1–39.
Rawlings, C.A. (1986b) Radiographic and electrocardiographic diagnoses. In: Rawlings, C.A. (Ed.) Heartworm disease in dogs and cats. Philadelphia, PA: W.B. Saunders, pp. 57–90.
Rawlings, C.A., Raynaud, J.P., Lewis, R.E. & Duncan, J.R. (1993) Pulmonary thromboembolism and hypertension after thiacetarsamide vs melarsomine dihydrochloride treatment of *Dirofilaria immitis* infection in dogs. *American Journal of Veterinary Research*, **54**, 920–925.
Reinero, C., Visser, L.C., Kellihan, H.B., Masseau, I., Rozanski, E., Clercx, C. et al. (2020) ACVIM consensus statement guidelines for the diagnosis, classification, treatment, and monitoring of pulmonary hypertension in dogs. *Journal of Veterinary Internal Medicine*, **34**, 549–573.
Savadelis, M.D., Ohmes, C.M., Hostetler, J.A., Settje, T.L., Zolynas, R., Dzimianski, M.T. et al. (2017) Assessment of parasitological findings in heartworm-infected beagles treated with Advantage Multi® for dogs (10% imidacloprid + 2.5% moxidectin) and doxycycline. *Parasites & Vectors*, **10**, 245.
Travell, J. (1955) Factors affecting pain of injection. *Journal of the American Medical Association*, **158**, 368–371.
Vezzoni, A., Gechi, C. & Raynaud, J.P. (1992) Adulticide efficacy of RM340 in dogs with mild and severe natural infections. In: Proceedings of the 1992 American Heartworm Synposium. Batavia, IL: American Heartworm Society, p. 231.
Wallis, C., Saito, E.K., Salt, C., Holcombe, L.J. & Desforges, N.G. (2021) Association of periodontal disease with breed size, breed, weight, and age in pure-bred client-owned dogs in the United States. *Veterinary Journal*, **275**, 105717.
Yoon, W.K., Kim, Y.W., Suh, S.L., Choi, R., Lee, S.-G. & Hyun, C. (2017) Evaluation of cardiopulmonary and inflammatory markers in dogs with heartworm infection during treatment with the 2014 American Heartworm Society recommended treatment protocol. *Parasites & Vectors*, **10**(Supplement 2), 535.