Veterinary Parasitology 271 (2019) 54–56

Contents lists available at ScienceDirect

Veterinary Parasitology

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

Research paper

Variation of the adulticide protocol for the treatment of canine heartworm infection: Can it be shorter?

E. Carretón[a,*], Y. Falcón-Cordón[a], S. Falcón-Cordón[a], R. Morchón[b], J.I. Matos[a], J.A. Montoya-Alonso[a]

[a] Internal Medicine, Faculty of Veterinary Medicine, Research Institute of Biomedical and Health Sciences (IUIBS), University of Las Palmas de Gran Canaria, Las Palmas de Gran Canaria, Spain
[b] Group GIR of animal and human dirofilariosis, Laboratory of Parasitology, Faculty of Pharmacy, University of Salamanca, Salamanca, Spain

ARTICLE INFO

Keywords:
Heartworm
Adulticide treatment
Melarsomine dihydrochloride
Canine dirofilariosis

ABSTRACT

The treatment of canine heartworm has been modified over the years, adding improvements for greater efficacy, safeness and better prognosis. Currently, the recommended adulticidal protocol consists of the administration of three doses of melarsomine dihydrochloride, preceded by the administration of macrocyclic lactones over two to three months. The objective of this study was to evaluate a variation of the adulticide protocol of heartworm in 76 dogs infected by *Dirofilaria immitis*, which consists of the pre-administration of macrocyclic lactones (ivermectin) during a single month. On the day of diagnosis, presence of circulating microfilariae was determined and an echocardiography was performed to assess the parasite burden. Treatment began on day 0, with doxycycline for 30 days (10 mg/kg BID) and monthly ivermectin (6mcg/kg). On day 30, the first dose of melarsomine dihydrochloride was administered, followed by a second and third dose on days 60 and 61, respectively. On day 90, the dogs were examined and discharged. Six months after the last dose, all dogs were negative to the presence of antigens and amicrofilaremic. Also, 38.1% of animals were evaluated by echocardiography, showing absence of adult parasites. It is considered that the ineffectiveness of melarsomine against worms < 4 months should be avoided by the previous administration of macrocyclic lactones for two to three months, killing larvae < 2 months while older filariae are allowed to mature to be susceptible to melarsomine dihydrochloride. With this protocol, this gap would be covered for the 2nd and 3rd injections, when worms would be four months and older. In addition, there is evidence that melarsomine is effective against worms under four months and macrocyclic lactones have some efficacy against heartworms older than two months. This modification allows a faster elimination of heartworms and a better compliance from the owners of the infected dogs.

1. Introduction

Canine heartworm (*Dirofilaria immitis*) is a severe and potentially mortal disease in dogs. Despite the frequent campaigns carried out with the aim of preventing infection, and that both veterinarians and owners are highly aware of the importance of prevention and the consequences of infection, especially in endemic areas, canine heartworm is considered an emerging disease and, in recent years, new canine heartworm infections have been reported in areas that, until then, were considered free of the disease (Genchi et al., 2011; Morchón et al., 2012; Miterpáková et al., 2018).

The treatment of canine heartworm has been modified over the years, adding improvements aimed to achieve greater efficacy and better prognosis. Currently, the only authorized adulticide treatment for the infection consists on the administration of an arsenical agent with adulticidal properties, melarsomine dihydrochloride, which causes the death of adult worms lodged in the pulmonary arteries of the host. The adulticidal protocols recommended by the American Heartworm Society and the European Society of Dirofilariosis and Angiostrongylosis comprise the administration of three doses of melarsomine dihydrochloride, preceded by the administration of macrocyclic lactones over two to three months (European Society of Dirofilariosis and Angiostrongylosis, 2017; American Heartworm Society, 2018). The objective of this pre-treatment prior to the administration of the adulticide drug was to cover the so-called susceptibility gap. This is defined as the time interval in which some heartworm stages are not susceptible to treatment with either macrocyclic lactones or melarsomine dihydrochloride, since melarsomine does not

* Corresponding author at: Internal Medicine, Faculty of Veterinary Medicine, University of Las Palmas de Gran Canaria, 35413, Arucas, Las Palmas, Spain.
E-mail address: elena.carreton@ulpgc.es (E. Carretón).

https://doi.org/10.1016/j.vetpar.2019.06.006
Received 29 April 2019; Received in revised form 6 June 2019; Accepted 10 June 2019

seem to show adulticidal activity against heartworm younger than four months of age and macrocyclic lactones lose effect on worms older than two months (Bowman and Drake, 2017). This interval consists of two to three months prior the administration of melarsomine dihydrochloride, in which the administration of macrocyclic lactones will avoid new infections and eliminate susceptible larvae while allowing the older worms to grow enough to be susceptible to melarsomine.

However, the long duration of this protocol causes, on the one hand, between 10–15% of owners to abandon the treatment before initiating the administration of melarsomine dihydrochloride (Carretón, 2019, personal communication); in some cases, it may be due to the relative improvement of clinical signs due to the elimination of microfilariae, larvae and *Wolbachia* and the loss of contact with the veterinarian for a long period of time. On the other hand, during that lapse of time, the adult parasites continue in the pulmonary arteries, worsening the vascular damage and aggravating any deterioration at cardiopulmonary or renal level. Also, during that interval of time, the risks of spontaneous and fatal thromboembolism continue, especially if exercise restriction is not being performed (McCall et al., 2008; Carretón et al., 2017).

Furthermore, there is evidence that melarsomine dihydrochloride has properties against immature worms from two to four months old as well as monthly administration of some macrocyclic lactones (i. e. ivermectin, selamectin, milbemycin oxime) and may be total or partially effective against worms older than two months (McCall, 2005; McCall et al., 2010).

Therefore, given the possibility to eliminate the susceptibility gap with a modified treatment, the objective of this study was to evaluate the efficacy of a variation of the adulticide protocol of canine heartworm, which consists of the pre-administration of macrocyclic lactones during a single month.

2. Materials and methods

This study involved 76 dogs living in a hyperendemic area of heartworm disease (Montoya-Alonso et al., 2016) and naturally infected by *D. immitis*. Animals were attended at the Veterinary Medicine Service of the University of Las Palmas de Gran Canaria where diagnosis of heartworm infection was carried out by the detection of circulating antigens of the parasite using a commercial kit (Urano Test Dirofilaria®, Urano Vet SL, Barcelona, Spain).

Of them, 32 were male and 44 were female and the age range varied from 1 to 9 years (mean age 4.7 years); 29 were mixed-breed dogs and 36 were pure-bred dogs. None of the dogs had received previous heartworm prophylaxis or adulticide treatment against the disease, nor presented previous or concomitant pathologies.

All the animals underwent physical examination, and imaging tests were carried out by performing thoracic radiography and echocardiography. By means of the ultrasound exam, the presence of parasites and worm burden were estimated following previously established guidelines (Venco et al., 2003). In addition, on the day of diagnosis, the dogs were evaluated for the detection of circulating microfilariae using a modified Knott test.

The dogs began treatment on day 0 with the administration of doxycycline for 30 days (10 mg/kg BID) and monthly ivermectin (6mcg/kg). On day 30, all dogs were treated with the first intramuscular injection of melarsomine dihydrochloride (2.5 mg/kg), followed on day 60 by a second injection, and a third injection on day 61. Moderate exercise restriction was recommended from day 0 to day 30 and significant restriction was strongly advised from day 30 until at least one month after the last injection of melarsomine dihydrochloride. On day 90, all patients were examined; they underwent a physical examination, while Knott's test, echocardiographic examination, and chest radiographs were also performed. If no clinical signs were present and the echocardiography showed no presence of adult parasites, the animals were discharged. Monthly heartworm prophylaxis was not interrupted during the study.

Six months after completion, an antigen test was done to confirm the adulticide efficacy in all patients; furthermore, echocardiographic exam was performed to 33 (43.4%) of the dogs to determine the presence or absence of adult parasites.

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-2015/02) and was executed in accordance with the current European legislation on animal protection.

3. Results

On day 0, 48.7% of the dogs presented microfilaremia and 51.3% were amicrofilaremic. During the echocardiographic examination, 82.9% (63/76) of the animals presented a low parasitic burden and 17.1% (13/76) had a high burden, according to Venco et al. (2003). Age was similar between those with low (mean: 4.7 years) and high burden (mean: 4.4 years).

Symptoms compatible with heartworm disease were considered in animals showing one or more of these signs: cough, dyspnea, cachexia, exercise intolerance, syncope, ascites, hemoptysis, lung crackles, right-sided heart murmur or split second heart sound). Symptoms were present in 60.5% (46/76) of the dogs, being 55.5% (35/63) in dogs with low parasite burden and 84.6% (11/13) of dogs with high burden. Mean age of asymptomatic dogs was 4.05 years and 5.1 years for the symptomatic animals.

All the dogs finished the adulticide treatment without showing severe or moderate side effects. On day 90, all patients were discharged due to the absence of adult worms by echocardiography, as well as the absence of microfilariae and clinical signs.

Six months after the third dose of melarsomine, all dogs showed absence of circulating antigens in the commercial test as well as absence of microfilariae. No adult worms were observed in those dogs which underwent echocardiographic examination.

4. Discussion

The adulticide treatment of heartworm disease has evolved over the years, replacing thiacetarsamide sodium, used in the 1980s, by melarsomine dihydrochloride in the 1990s. It was demonstrated that the latter was a more effective, safer and easier product to administer (Rawlings et al., 1993; Maksimowich et al., 1997). In addition, the protocols with melarsomine dihydrochloride were subsequently modified, changing from a two-dose protocol (2.5 mg/kg administered IM, 24 h apart), to another of three doses (a single dose followed 30 days later by two doses, separated by 24 h). This modified protocol proved to have greater adulticide efficacy, as well as greater safety for the patient, because the risk of severe pulmonary thromboembolism was reduced due to a staggered death of adult parasites (Carretón et al., 2014; American Heartworm Society, 2018).

Until recently, it was considered that the ineffectiveness of melarsomine against worms under four months should be avoided by the previous administration of macrocyclic lactones. Thus, the so-called susceptibility gap was established as a period of two to three months before administering melarsomine dihydrochloride. This was indicated to eliminate larvae less than two months old, susceptible to macrocyclic lactones, while allowing the rest of the worms to grow until reaching an age old enough ($\geq$4 months) to be considered susceptible to melarsomine dihydrochloride. However, a recent review article showed evidence that melarsomine may be effective against worms under four months, which called into question the susceptibility gap and, in fact, this concept has been recently removed from the latest guidelines (Bowman and Drake, 2017; American Heartworm Society, 2018).

In the protocol proposed in the present study, it is taken into consideration that the susceptibility gap, if still considered, would be covered at the time of the second and third doses of melarsomine, since

the worms that were not susceptible to the macrocyclic lactones on day 0 – and, therefore, with an age equal to or greater than two months - would be old enough to be susceptible to the adulticide drug on day 60, when they were ≥4 months old. In addition, it has been reported that the continued monthly administration of some macrocyclic lactones (i.e. ivermectin, selamectin, milbemycin oxime) has high efficiencies, between 96.7% and 98.5% against 3-month heartworms. Furthermore, it has been described efficiencies of 41.4% (milbemycin oxime) and 95.1% (ivermectin) against 4-month parasites after 12 months of administration (McCall, 2005). Furthermore, two doses of melarsomine 24 h apart showed 100% efficacy against 2-month old worms in five dogs experimentally infected, suggesting that melarsomine dihydrochloride would have efficacy against younger parasites (McCall et al., 2010), so the susceptibility gap may be disregarded (Bowman and Drake, 2017).

Although initiating adulticide treatment in the moment of the diagnosis was considered a plausible protocol (Bowman and Drake, 2017), the previous administration of doxycycline to reduce the population of *Wolbachia* is recommended. *Wolbachia* is a bacterium that plays an important role in the pathogenesis of the disease, and generates the release of proinflammatory and chemotactic cytokines, which induce infiltration cellular and amplification of the inflammatory response (Kramer et al., 2008). The reduction of the levels of *Wolbachia* in *D. immitis* prior the adulticide treatment has demonstrated better adulticide results with less inflammatory reaction in dogs and lower risk of fatal pulmonary thromboembolisms, and, therefore, could considerably improve the prognosis of the dogs that are receiving the treatment (Bazzocchi et al., 2008; Kramer et al., 2011). In the protocol of the present study, 30 days of doxycycline were administered together with the first dose of ivermectin with the aim to eliminate *Wolbachia* prior the first adulticide dose of melarsomine dihydrochloride.

Moreover, starting soon after the arrival of the worms to the pulmonary vasculature, its presence causes endothelial damage with villous proliferation of the intima of the arteries, vascular and pulmonary inflammation, pulmonary hypertension, disruption of vascular integrity, and fibrosis (Bowman and Atkins, 2009; Simón et al., 2012). The more time worms spend in the pulmonary vasculature, the greater the damage that their presence can cause. Also, the spontaneous death of worms can cause acute and fatal pulmonary thromboembolisms (McCall et al., 2008). While during the adulticide treatment the exercise restriction in patients is strict, during the two to three months prior the administration of melarsomine, this restriction is frequently neglected and compliance in this recommendation may be lost due to the long period of time required. Hence, this modification of the adulticidal protocol would allow an earlier elimination of the adult heartworms of the pulmonary vasculature, minimizing the damages and risks that its presence could entail, and increasing owner compliance. To determine if the adherence of the owners to the treatment will be greater by using this protocol, it is necessary to perform new independent studies.

The results of this work show that the evaluated adulticide treatment protocol was effective, regardless the microfilaremic status and the parasite burden detected by echocardiography, so it could be validated for its use in dogs with heartworm. Thus, this modification of the adulticidal protocol would improve prognosis, owner compliance and minimize the damage of the pulmonary vasculature that its presence could have caused.

References

American Heartworm Society, 2018. Current Canine Guidelines for the Prevention, Diagnosis and Management of Heartworm (*Dirofilaria Immitis*) Infection in Dogs (2018). Available at: http://www.heartwormsociety.org (Accessed 24.04.19). .

Bazzocchi, C., Mortarino, M., Grandi, G., Kramer, L.H., Genchi, C., Bandi, C., Genchi, M., Sacchi, L., McCall, J.W., 2008. Combined ivermectin-doxycycline treatment has microfilaricidal and adulticide activity against D. immitis in experimentally infected dogs. Int. J. Parasitol. 38, 1401–1410.

Bowman, D.D., Atkins, C.E., 2009. Heartworm biology, treatment, and control. Vet. Clin. North Am. Small Anim. Pract. 39, 1127–1158 vii.

Bowman, D.D., Drake, J., 2017. Examination of the "susceptibility gap" in the treatment of canine heartworm infection. Parasit. Vectors 10 (Suppl 2), 513.

Carretón, E., Morchón, R., Montoya-Alonso, J.A., 2017. Cardiopulmonary and inflammatory biomarkers in heartworm disease. Parasit. Vectors 10 (Suppl 2), 534.

Carretón, E., Morchón, R., Simón, F., Juste, M.C., González-Miguel, J., Montoya-Alonso, J.A., 2014. Evaluation of cardiopulmonary biomarkers during classic adulticide treatment versus the American Heartworm Society recommended treatment protocol in dogs infected by Dirofilaria immitis. Vet. Parasitol. 206, 55–59.

European Society of Dirofilariosis and Angiostrongylosis, 2017. Guidelines for Clinical Management of Canine Heartworm Disease. Available at: http://www.esda.vet (accessed 24.04.19). .

Genchi, C., Mortarino, M., Rinaldi, L., Cringoli, G., Traldi, G., Genchi, M., 2011. Changing climate and changing vector-borne disease distribution: the example of Dirofilaria in Europe. Vet. Parasitol. 176, 295–299.

Kramer, L., Grandi, G., Leoni, M., Passeri, B., McCall, J., Genchi, C., Mortarino, M., Bazzocchi, C., 2008. Wolbachia and its influence on the pathology and immunology of Dirofilaria immitis infection. Vet. Parasitol. 158, 191–195.

Kramer, L., Grandi, G., Passeri, B., Gianelli, P., Genchi, M., Dzimianski, M.T., Supakorndej, P., Mansour, A.M., Supakorndej, N., McCall, S.D., McCall, J.W., 2011. Evaluation of lung pathology in *Dirofilaria immitis*-experimentally infected dogs treated with doxycycline or a combination of doxycycline and ivermectin before administration of melarsomine dihydrochloride. Vet. Parasitol. 176, 357–360.

Maksimowich, D.S., Bell, T.G., Williams, J.F., Kaiser, L., 1997. Effect of arsenical drugs on in vitro vascular responses of pulmonary artery from heartworm-infected dogs. Am. J. Vet. Res. 58, 389–393.

McCall, J.W., 2005. The safety-net story about macrocyclic lactone heartworm preventives: a review, an update, and recommendations. Vet. Parasitol. 133, 197–206.

McCall, J.W., Genchi, C., Kramer, L.H., Guerrero, J., Venco, L., 2008. Heartworm disease in animals and humans. Adv. Parasitol. 66, 193–285.

McCall, J.W., Kramer, L., Genchi, C., Guerrero, J., Dzimianski, M., Supakorndej, P., Mansour, A., McCall, S.D., Supakorndej, N., Grandi, G., Carson, B., 2010. Effects of melarsomine dihydrochloride on two-month-old infections of dirofilaria immitis and brugia pahangi in dogs with dual infections. State of the Heartworm 2010 Symposium, 16–18 April. American Heartworm Society (Abstract), Memphis, TN.

Miterpáková, M., Valentová, D., Čabanová, V., Berešíková, Ľ., 2018. Heartworm on the rise-new insights into *Dirofilaria immitis* epidemiology. Parasitol. Res. 117, 2347–2350.

Montoya-Alonso, J.A., Carretón, E., Morchón, R., Silveira-Viera, L., Falcón, Y., Simón, F., 2016. The impact of the climate on the epidemiology of *Dirofilaria immitis* in the pet population of the Canary Islands. Vet. Parasitol. 216, 66–71.

Morchón, R., Carretón, E., González-Miguel, J., Mellado-Hernández, I., 2012. Heartworm disease (*Dirofilaria immitis*) and their vectors in Europe - new distribution trends. Front. Physiol. 3, 196.

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. Am. J. Vet. Res. 54, 920–925.

Simón, F., Siles-Lucas, M., Morchón, R., González-Miguel, J., Mellado, I., Carretón, E., Montoya-Alonso, J.A., 2012. Human and animal dirofilariasis: the emergence of a zoonotic mosaic. Clin. Microbiol. Rev. 25, 507–544.

Venco, L., Genchi, C., Vigevani Colson, P., Kramer, L., 2003. Relative utility of echocardiography, radiography, serologic testing and microfilariae counts to predict adult worm burden in dogs naturally infected with heartworms. In: Seward, R.L., Knight, D.H. (Eds.), Recent Advances in Heartworm Disease, Symposium'01. American Heartworm Society, Batavia, IL, pp. 111–124.