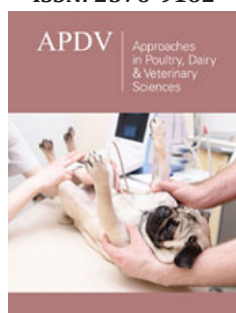


Seronegative Results After the Administration of the Combination of Doxycycline and Moxidectin in Infected Dogs by *Dirofilaria Immitis*

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Abstract

Dirofilariasis or canine heartworm infection is a serious and potentially fatal disease caused by the filarial nematode *Dirofilaria immitis*, a parasite transmitted through infected mosquitoes. Environmental and climatic changes, as well as transportation of animals and materials have increased heartworm infection potential making this parasitosis to present now a nearly worldwide distribution. Clinically healthy dogs from various areas of the prefecture of Thessaloniki, North Greece, were presented for routine serological examination against common parasitic diseases during the first half of 2021. 113 of them were found positive for dirofilariasis using the serological commercial kit Pet Check® (IDEXX), for the detection of adult *D. immitis* antigens. All 113 naturally infected dogs that were included in this study followed an alternative therapeutic protocol that consisted of doxycycline 10mg/kg q 12h PO for one month in combination with the administration of moxidectin (2.5mg/kg)+imidacloprid (10mg/kg) applied topically q 30d, for 12 months. There is no doubt that the protocol for treating canine dirofilariasis by using melarsomine is the first choice of treatment. However, in some countries this drug is not available. Subsequently clinicians are indirectly forced to suggest alternative treatments to improve and protect the health of infected dogs. The therapeutic protocol used in this study, where doxycycline is administered in combination moxidectin, has been shown to be successful in producing seronegative results for the specific antigens of *D. immitis* in all treated dogs after 12 months of administration.

Keywords: Dog; *Dirofilaria*; Doxycycline; Moxidectin; Treatment

Introduction

Dirofilariasis or canine heartworm disease is a serious and potentially fatal condition caused by the filarial nematode *Dirofilaria immitis* (*Filarioidea: Onchocercidae*). This vector born parasite infects domestic and wild canids, [1] but can also infect felids, ferrets and very rarely, humans [2-4]. Canids are the main reservoir of the infection [1] and mosquitoes, with more than 30 species of them, the only intermediate host and vector of dirofilariasis [1]. Environmental and climatic changes, as well as transportation of animals and materials have increased heartworm infection potential resulting in a nearly worldwide distribution of this parasitosis [5]. Dirofilariasis has a high prevalence in America [6,7], Europe and southeastern Asia, and is increasingly reported in Africa [8]. Some new reports state indigenous cases and prevalence of dirofilariasis in dogs from areas of Europe that were not previously enzootic [9]. Humans can also be infected with *D. immitis*, but infections are rare, with fewer than 100 cases reported in the USA over the last 60 years [10].

Adult parasites may live up to 5-7 years and are found in the heart, pulmonary artery and adjacent large blood vessels of infected dogs, causing severe damage. Females produce

millions of offspring called microfilaria. These live mainly in small vessels without causing any clinical sign. Microfilariae from infected dogs are included in a blood meal of a female mosquito and are ingested. These develop further for 10 to 30 days in the mosquito's gut, depending on the climate conditions and then move in its mouthparts, where they are infective larvae. They can complete their life cycle in about 7-9 months, after they enter into a new dog by the mosquito's bite [11,12]. Other rare hosts of dirofilariasis, such as cats and ferrets, have occasionally low-level, transient microfilaremia and may serve as a limited source of infection for mosquitoes [2].

Materials and Methods

In this study, clinically healthy dogs from areas of the prefecture of Thessaloniki, northern Greece, were presented in various veterinary clinics, during the first half of 2021, for routine serological examination against dirofilariasis. A blood sample was collected in EDTA tube from each dog and screened with a commercial antigen immunochromatographic test system for the detection of circulating heartworm antigen, according to the manufacturer's instructions (Pet Check® IDEXX). This test documents high sensitivity (99.0%) and specificity (99.3%). The current generation of *D. immitis* antigen tests identify infections consisting of at least one mature female worm and are nearly 100% specific [10,13,14]. 113 (64 males and 49 females) of the examined dogs were tested positive. All positive dogs were characterized stage I-II and none of these dogs recorded with pulmonary hypertension or severe symptoms. The great majority originated from rural areas outside the city's urban complex where there are suitable environmental conditions for the widespread spread of mosquitoes such as rivers and rice crops. The purpose of this study is to document the efficacy of the use of doxycycline in combination with the periodic use of moxidectin for at least 12 months for the treatment of canine dirofilariasis. This alternative protocol was granted, with the consent of the pets' owners, due to the lack of melarsomine in our country, as in other countries worldwide [15,16]. The treatment protocol consisted of the administration of glucocorticosteroids in anti-inflammatory doses (prednisone at 0.5mg/kg PO BID for the first week, 0.5mg/kg PO SID for the second week, 0.5mg/kg PO EOD for the third and fourth week), doxycycline (10mg/kg PO BID for 4 weeks) and topical application of moxidectin (2.5mg/kg)+imidacloprid (10mg/kg) (spot on) every 30 days for 12 months. Cage rest and activity restriction were strongly advised for at least 8 weeks.

Results

At the end of the 12 month period, a blood sample was collected from each treated dog and tested using again the kit Pet Check® (IDEXX). All samples tested negative for *D. immitis* antigens. Other studies report similar results [16,17].

Discussion

Without a doubt, the protocol for treating canine dirofilariasis that includes melarsomine is the first choice of treatment. It is the safest, most efficacious, and the only protocol currently recommended for the treatment of canine heartworm disease,

also suggested by the American Heartworm Society and the Companion Animal Parasite Council. It includes the administration of 3 doses of the arsenical drug melarsomine dihydrochloride, combined with doxycycline and a macrocyclic lactone [18,19]. On the other hand, in some countries melarsomine is not available. Subsequently clinicians are indirectly forced to suggest other alternative treatments to improve and protect the health of infected dogs. To this direction the present study tries to fill the void that the unavailability of melarsomine creates. Glucocorticosteroids were administered in all dogs at anti-inflammatory doses. They help controlling clinical signs of pulmonary thromboembolism [20] and are indicated especially in cases of high microfilaria burdens to minimize potential anaphylactic reactions after the administration of a microfilaricide [1].

Doxycycline is administered at 10mg/kg q 12h PO for one month [18,21,22]. It acts against *Wolbachia* (Rickettsiales), an intracellular endo-symbiotic gram-negative bacteria [23,24], vital for the worms and sensitive to tetracycline antibiotics [25]. McCall et al. [26] reported that its administration during the first or second month after experimental dirofilaria infection was lethal to third and fourth stage larvae. Moreover, in dogs with adult infections, doxycycline gradually suppressed microfilariae [27,28]. Data also suggest that *Wolbachia* counts remain low for at least 12 months after doxycycline administration [25]. Microfilariae ingested by mosquitoes from doxycycline-treated dogs developed into third-stage larvae that appeared to be normal in appearance and motility, but could not develop into adult worms, thus reducing the risk of seizures [28,29]. Macrocyclic lactones have been successfully used for the prevention of heartworm disease for over 30 years [30,31]. They act at glutamate-gated chloride ion channels precipitating hyperpolarization, paralysis, and death of heartworms [32,33]. Other studies refer that some macrocyclic lactones, such as ivermectin and moxidectin, combined with doxycycline suppress embryogenesis, weaken the adult *D.immitis* and have adulticidal activity [28,34-36].

Moxidectin is more lipophilic than other macrocyclic lactones with a higher volume of distribution, longer half-life and slower elimination [37-39]. Its concentrations after topical dosing remain high for at least 28 days at the labeling dose, with steady state achieved after four monthly treatments [30]. It should be noted that the adult killing effect of macrocyclic lactones has been shown to require more than 2 years of continuous administration before 95% of adult worms are eliminated [40]. Throughout this period, the pathology of the infection would remain and probably continue to evolve [41-43] and test results may be false negative because the treatment did not eliminate all life stages that are still developing.

Conclusion

Alternative therapy of natural dirofilariasis of dogs using the combination of glucocorticosteroids, doxycycline and moxidectin+imidacloprid (for 12 months), in cases where melarsomine is not available, has been shown to be successful in producing seronegative results in all treated dogs. Nevertheless, it should be noted that this combination does not kill effectively

all parasitic stages and, although dogs test seronegative, parasites continue to exist and cause damage to tissues. Thus, further research is needed to investigate potential consequences on the overall health and survival of canine patients due to this extended presence of *D. immitis* in the animal's body, compared with the standard therapeutic protocol that includes melarsomine. Suitable candidates for this therapeutic protocol, adverse reactions and special cautions remain to be clarified.

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