Macrocyclic Lactones and *Dirofilaria immitis* Microfilariae

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Although there has been extensive veterinary focus on both the effectiveness of macrocyclic lactones for heartworm prevention in dogs and their adulticidal effects, little attention has been directed to their effects on heartworm microfilariae. With routine use of macrocyclic lactones, in some cases knowingly, in dogs with existing heartworm infections, veterinarians should recognize the benefits, and possible complications, arising from this behavior. Macrocyclic lactones remain our only class of heartworm prevention available, and preserving their effectiveness is critical. Drugs in this class share common traits: there are currently no Food and Drug Administration—approved microfilaricides in the US marketplace, but because all macrocyclic lactones have microfilaricidal properties (to varying degrees), they are widely used by veterinarians for this purpose. Originally formulated to be used in dogs without patent heartworm infections, all have been demonstrated as safe to use at label doses, and higher, in microfilaremic dogs. All of the product labels indicate that dogs should be tested for heartworm infection before starting preventive therapy. Although microfilaricidal, microfilariae reduction may take many months to occur, and some dogs may never clear. The effects of macrocyclic lactones on the numbers of circulating microfilariae may be due to several different underlying causes (i.e., direct effect on the nervous system, affecting stages found in the uterus of the female worms), but the details of all mechanisms by which microfilariae are killed and/or cleared in dogs treated with macrocyclic lactones have not yet been fully elucidated. Some 10% to 20% of heartworm-infected dogs that begin monthly heartworm preventive treatment without adulticide therapy will have the continued and persistent presence of circulating microfilariae, and the concern is that this may be selecting for resistance to these molecules. The veterinary literature now includes evidence of increased genotypic homozygosity in specific dogs in one area of the country for a marker gene associated with macrocyclic lactone resistance in nematodes of ruminants. This article will review the biology of microfilariae, as well as the evolution of diagnostic testing for heartworm infection. The effects of macrocyclic lactones on microfilaria behavior and survival will be discussed, as well as the use and effects of macrocyclic lactones in microfilaremic dogs, with or without adulticide treatment. The effect of doxycycline on heartworm microfilariae, optimal testing methodologies, and verification of effective clearance of microfilariae after adulticide treatment and microfilaricidal therapy so that dogs do not remain a potential source of infection for other dogs are all covered.

Keywords: macrocyclic lactones, heartworm, preventives, microfilaria, chemoprophylactics, adulticides, anthelmintic

In the last few years, there has been a noted increase in the lack of efficacy reports (LOEs) for different heartworm preventives.[1] Also, there have been a number of reports from clinicians in the field and various veterinary parasitologists at different veterinary colleges around North America on the inability of macrocyclic lactones to clear microfilariae from heartworm—antigen negative dogs after adulticide therapy; in some of these cases even after the treated dogs are administered dosages of ivermectin exceeding 200 μg/kg.[2] Thus, it seemed the right time to review the biology of heartworm infection, the production of microfilariae, and the published information on the effects of macrocyclic lactones on microfilaria behavior and survival.

The Filarioidea is a large superfamily of nematode parasites within the order Spirurida that are parasites of the tissues and tissue spaces of all vertebrates other than fish.[3] These worms are all transmitted by hematophagous arthropods. Among the Onchocercidae, the family in which the canine heartworm *Dirofilaria immitis* is placed (Nematoda: Spirurida, Filarioidea, Onchocercidae, Dirofilariinae), the different species have a blood- or skin-inhabiting microfilarial stage that is transmitted between hosts by arthropod-intermediate hosts that create lesions or pierce the skin to suck blood, providing access to the microfilariae. The microfilarial stage is particular to this group of worms and is crit-

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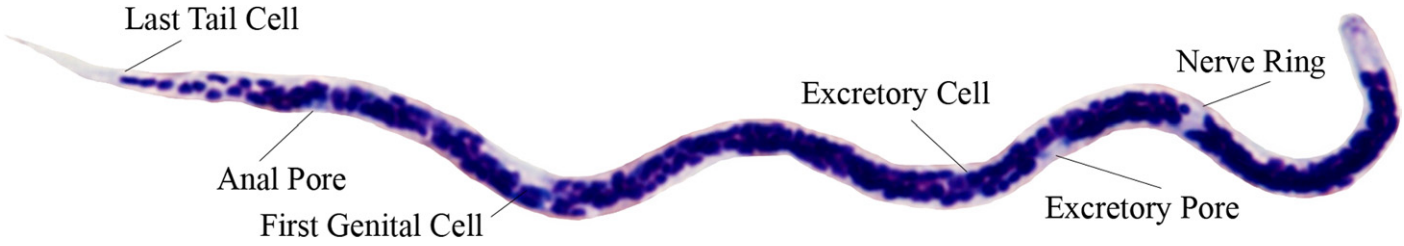

Figure 1. Microfilaria of *Dirofilaria immitis*, Giemsa stain, 40×. Showing the position of the various morphologic landmarks: nerve ring, excretory pore, excretory cell, G1 cell, and the last tail cell.

ical for transmission by the blood-feeding arthropod-intermediate host. "The microfilaria is essentially a highly motile, thread-like prelarva that in some species retains the egg membrane as a sheath, i.e., it becomes a sheathed form, whereas in others the microfilaria ruptures the egg membrane to become a naked 'unsheathed' form. The egg membrane is shed, or not, usually while the microfilariae are still in the uterus. On being extruded by the female, the microfilariae enter the blood or lymphatic vessels, and while circulating in peripheral blood or moving about in the cutaneous tissues they are ingested by blood sucking arthropods."[4]

The microfilariae that the canine heartworm produces circulate in the blood, allowing their access to the vector, a blood-feeding female mosquito. The microfilaria of *Dirofilaria immitis* can usually be identified morphologically by careful examination of microfilarial structure and morphometrics (Fig 1). In the United States, a few other microfilariae have been identified in the blood of dogs.[5] The microfilariae of the small filarioid that lives in the subcutaneous tissues of dogs, *Acanthocheilonema* (*Dipetalonema*) *reconditum*, are sometimes found in the blood. This worm is transmitted between dogs by the bites of fleas or the sucking louse *Heterodoxus spiniger*, and infections seem to be rarer now than in the past because of improved louse and flea control. Other microfilariae that have been found in dogs in the United States include those of the "Irish" *Dipetalonema* that was first found in the blood of dogs from southern Ireland that had been imported into Florida. The microfilariae of this *Dipetalonema* species are shorter than those of *A. reconditum* and *Dirofilaria immitis*. The tissue-dwelling *Dirofilaria striata* of the bobcat has also been reported on rare occasions from dogs in Florida, and the microfilaria of this species are characterized by 2 prominent nuclei in the anterior end that appear on appropriately stained preparations. Another rare finding has been microfilariae of a *Dirofilaria* species that are similar to *Dirofilaria striata* in that they are longer than *Dirofilaria immitis*, but they lack the cephalic nuclei of *Dirofilaria striata*. There are a few other filarioid nematodes found in dogs around the world.[3] It appears that the fairly common skin-dwelling *Dirofilaria repens* of dogs of southern Europe and much of the rest of the world has not become indigenous in the United States, or at least, microfilariae have not yet been found in dogs that do not have a history of travel to endemic regions. Aside from *Dirofilaria repens*, there are species of *Brugia* that live in the lymphatics of dogs around the world.[6] This includes *B. patei* in dogs in Sri Lanka, *B. pahangi* in dogs and cats in Africa, and *B. malayi* in dogs, cats, and people in India and Africa. Also, there is *Dipetalonema dracunculoides*, which lives in the peritoneal cavity of dogs in North Africa and northern Kenya, and has microfilariae that circulate in the blood. *Dipetalonema grassi* is found in Italy, Kenya, and Brazil, but this skin-dwelling filariid has microfilariae that are found in the skin, and only very rarely in blood samples. Recently, a short and stubby microfilaria that had previously been called *Microfilaria auquieri* by Foley in 1921 was noted in the blood of dogs in India.[7,8] More and more commonly, specific identification of microfilariae can be performed with methods other than morphologic examination. The antigen tests that are used for *Dirofilaria immitis* are specific for this nematode relative to *A. reconditum* and *Dirofilaria repens*.[9,10] Also, it has become possible to identify the species of microfilaria present in a dog with molecular methods for specific identification.[10,11] Thus, positive identification of microfilariae in cases in which identification would be helpful for discrimination of infection relative to epidemiology, atypical case presentations, or inability to clear an infection or microfilariae after routine treatment regimens is much better now than it was only a few years ago.

The microfilariae of *Dirofilaria immitis* develop in the uterus of an adult female that has mated with a male worm, and it is suspected that females must mate repeatedly to continue to produce viable offspring throughout the course of their lives. In the uterus of the inseminated female, the microfilariae develop from eggs into stretched microfilariae. The microfilariae that leave the ovoviviparous female have hatched out of the very thin eggshells in which they developed and are hence unsheathed microfilariae. These microfilariae are made in very high numbers and circulate in the blood of the dog to allow for transmission through very small quantities of blood ingested by a feeding female mosquito.

In nature, dogs acquire and develop heartworm infections only after the delivery of third-stage larvae that have developed to the infective stage in a mosquito that ingested microfilariae in a previous blood meal. While the mosquito is feeding, the third-stage larva leaves the mosquito's mouthparts and enters the hole in the dog's skin made by the biting mosquito. The larval development within the mosquito is required for the heartworm to continue its development to