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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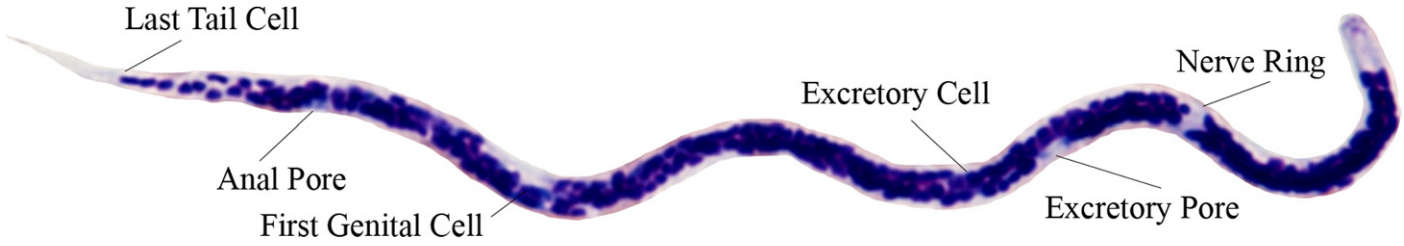

Figure 1. Microfilaria of *Dirofilaria immitis*, Giemsa stain, 40×. Showing the position of the various morphologic landmarks: nerve ring, excretory pore, excretory cell, G1 cell, and the last tail cell.

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

the adult stage. It is only the third-stage larva that can go on to develop into an adult heartworm.

Microfilariae can on occasion be found in dogs that were never infected with third-stage larvae from mosquitoes. Occasionally, microfilariae of *Dirofilaria immitis* can pass from a heartworm-infected dam to her pups through the placenta,[12] or microfilariae may be passed from infected donor dogs to recipient dogs during blood transfusion,[13] but these microfilariae cannot develop to adult worms. Because microfilariae remain as microfilariae unless they enter a mosquito, any microfilariae in the blood of a dog remain in limbo as the prelarval microfilarial stage until they either die of old age, are cleared from the blood by a host response or chemical treatment, or enter the body of a mosquito to continue development. Circulating microfilariae have been shown to live for up to 2[1/2] years after transfusion.[13] The long life of a circulating microfilaria creates the possibility that a dog's blood may contain microfilariae after the adult female worms that gave birth to them have died and disintegrated. The adult worms that produced the microfilariae may have died of natural causes, and thus, it is possible for dogs to be microfilaremic and antigen negative even without pharmaceutical intervention. Often, the adults will have been killed by adulticide therapy, and typically the drugs used for this purpose have little or no effect on the viability of the circulating microfilarial stage; thus, in this case also, it is possible for a dog to be microfilaremic when it is antigen negative.

Microfilarial and Antigen Detection in Heartworm-infected Dogs

There was a time when the diagnosis of heartworm infection was based on clinical signs in association with the presence of microfilariae in the blood using methods such as direct smears, concentration of microfilariae in lysed blood, examination of buffy coats in microfuge tubes, or the passage of lysed blood through various filters that would trap the microfilarial stage. For microfilariae to be detectable using these methods, the presence of 30 or more microfilariae per milliliter of blood for detection on a blood film was required, whereas as few as 1 microfilaria per milliliter could be detected by a well-done Knott's test with 1 mL of blood. Thus, most veterinary practices in heartworm-endemic areas had competent staff and methodology for the in-house diagnosis of microfilariae in dog blood. These methods worked well in most dogs, most of the time, but not in all cases.

Using the various methods of microfilarial detection, it became clear that there were a fairly high number of dogs that harbored adult heartworms and had no circulating microfilariae. These infections without demonstrable circulating microfilariae were called "occult infections," a term introduced by Dr. Ming Wong in 1977 for dogs that had cleared their infections under immunologic influence (Dr. Wong, personal communication). Later, the term came to be applied more generally to any infections without microfilariae, i.e., in cases when worms could not produce microfilariae (in single-sex or geriatric infections); when the microfilariae were cleared by the immune response of the infected dog; or when the circulating microfilariae in the blood were reduced or cleared by the use of drugs active on the microfilariae either in the blood or as they developed in the uterus of the female worm.[14] In some studies in which dogs were necropsied after blood had been examined for microfilariae, occult infections accounted for as many as one third of the dogs that were ultimately diagnosed with heartworm infections.[15] This was of clinical concern because it made it difficult to verify infections in dogs that had obvious clinical signs of heartworm disease, but in which the infection status of the dog could not be verified.

In more recent years, assays for circulating microfilariae have been replaced by antigen-detection tests. The creation of these tests was driven to a large extent by the need to have a means to diagnose the occult heartworm case, i.e., the case with clinical signs and no demonstrable microfilariae. This interest was also spurred by the intent to diagnose heartworm infections before patency.[16] The improved methods are now better, but still they cannot detect infected dogs until at least 5 months after infection. Antibody detection tests were also developed to aid in the detection of occult filariasis,[17] but because of the assumption that most dogs in heartworm-endemic areas were likely to have been exposed to third-stage larvae, the antibody-detection tests that were developed were fraught with many of the same difficulties that beset antibody-detection tests that are used in feline heartworm infections. The antibody indicates that worms have entered the dog and developed to some extent, but they were not able to easily distinguish active from past infections, or active infections from exposure to larvae that did not complete their maturation. Thus, the need for and the usefulness of the antigen-detection tests were demonstrated and accepted as the good means to identify the presence of living worms.

Even with the antigen-detection tests, it is still not possible to detect all worms in all heartworm-positive dogs. One specific problem remains: the prepatent infection. Prepatent infections differ from occult infections in that they are due to worms that have not yet reached maturity. After a dog is infected with heartworms from the bite of a mosquito, it takes about 6 to 7 months before mature worms are capable of producing circulating microfilariae. Thus, there are about 7 months in which dogs have prepatent infections, i.e., infection before microfilariae are detectable. As stated above, the antigen tests that were developed for the detection of occult infections are capable of detecting infections with female heartworms beginning in some cases as early as 5 months after infection. The larvae coming out of the mosquito spend some 2[1/2] to 3 months in the body tissues of the dog before they enter the pulmonary vasculature. Thus, young heartworms will spend some 2 to 4 months in the pulmonary arteries before they can be detected by antigen or microfilarial tests.

The current tests for circulating antigen are considered to be independent of the presence of circulating microfilariae in the blood.[18] The antigen-detection tests that are currently

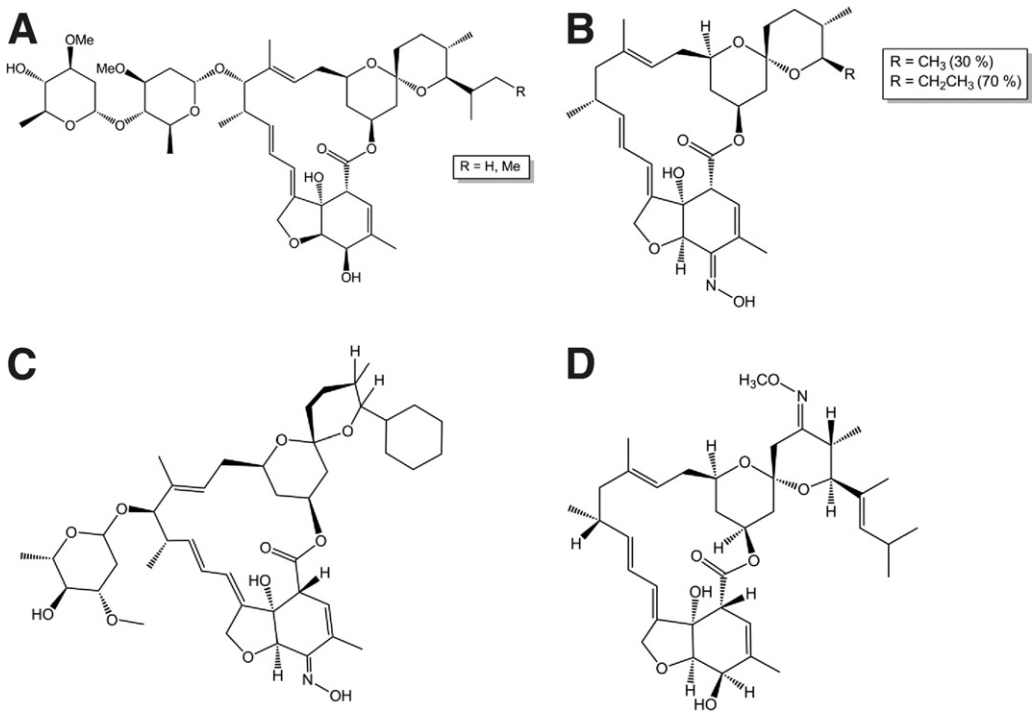

Figure 2. The macrocyclic lactones. (A) Ivermectin. (B) Milbemycin oxime. (C) Selamectin. (D) Moxidectin. Graphics prepared by Aleksandr Kalininsky.

used test for circulating antigens that come from female worms.[19] (A new test, the Anigen Rapid Canine Heartworm Antigen Test Kit from Modern Veterinary Therapeutics, LLC, claims to be able to detect male-only infections, but the data submitted for licensure to support this claim appear not to be verified elsewhere as of yet.) When the female worms are removed with adulticide therapy, the antigen is eventually cleared from the blood of the dog. In one study in Germany, of 19 dogs that were treated for their adult heartworm infections, only two still had circulating antigen in their blood nine weeks after treatment.[20] At 12 months after treatment, all dogs were antigen negative, 6 still have microfilariae, and only 3 dogs were found to have adult heartworms at necropsy. Thus, because of the independence of the circulating antigen-detection tests from the presence of circulating microfilariae, it is possible for dogs to be antigen negative after adulticide therapy while still having microfilariae present within the circulating blood.

Heartworm Prevention, Macrocyclic Lactones, and Safety

Heartworm prevention has focused on killing the third-stage larvae from the biting mosquito and the early fourth-stage larvae into which they develop. These stages have been the target of anthelmintic treatment because of the heightened vulnerability of the third-stage and young fourth-stage larvae to various anthelmintic products when compared with the susceptibility of the other stages in the life cycle. The 2 major groups of preventives that work very well on these stages are diethylcarbamazine citrate (DEC), a derivative of the heterocyclic piperazine, and the macrocyclic lactones. The currently approved macrocyclic lactones in the US veterinary marketplace include milbemycin oxime, ivermectin, moxidectin, and selamectin (Fig 2) in orally administered, topically administered, or injectable formulations. Dose titrations for preventive product development ignore the microfilarial stage and the later larval and adult stages. The goal is to deliver the minimal amount of product to the animal that will destroy the third-stage and early fourth-stage larvae that are trying to develop in the infected dog. Every macrocyclic lactone used in the veterinary market place has been found to be microfilaricidal to varying degrees. For the purpose of safety, these preventive products are examined for their effects on microfilariae and adult worms to determine what would occur if they were accidentally administered to dogs with patent infections. The concern is whether they would possibly cause dangerous clinical reactions in treated dogs because of massive numbers of dead or dying microfilariae or unexpected complications from adult worms dying in large numbers on administration.

In human onchocerciasis caused by the filarioid nematode *Onchocerca volvulus*, control took another approach. In the case of this infection, in which the most significant pathology is caused by the skin-dwelling microfilariae that cause corneal opacities and the disease "river blindness" and is caused by the difficulty in treating patients to kill the adult worms in their subcutaneous nodules, the decision was made to use macrocyclic lactones to suppress microfilariae in the infected population to stop transmission. This form of preventive

treatment in which adult worms are allowed to persist in the infected patient is termed altruistic prevention, i.e., preventing spread of transmission without removing the adult worms. Thus, large doses of ivermectin in a tablet formulation that suppress circulating microfilariae are administered one to several times a year and this administration of the macrocyclic lactone has the profound effect of suppressing microfilarial production by the female worms for several months without killing the adult worms. The goal of altruistic prevention is to keep people in a community on microfilarial suppression for 12 years and stop transmission until all the worms in the people in the community have died of old age. The great advantage of this microfilarial suppression is that it also curtails the blindness and skin disease that occurs in a patient because of the body's reaction to the microfilariae in the dermis (http://www.cartercenter.org/health/river_blindness/index.html).

Safety of Macrocyclic Lactones in Dogs with Patent Infections

In the development of the macrocyclic lactones for use as preventives for canine heartworm infection, a safety concern related to the potential untoward effects of heartworm preventives in animals was raised by existing knowledge about severe reactions occurring in dogs with circulating microfilariae treated with DEC. In 1947, when DEC was being developed into what was to become the daily heartworm preventive, reactions were seen in heartworm-infected dogs that were treated with this product.[21] Similar anaphylactic-like reactions and skin rashes due to the dying of the skin-dwelling microfilariae were known by 1948 to occur in human patients with *Onchocerca volvulus* treated with DEC.[22] Ultimately, DEC was developed into the daily preventive Filaribits (Filaribits Chewable Tablets, NADA 104-493), for heartworm prevention in dogs. Occasionally, the administration of the daily preventive to dogs with microfilariae unfortunately resulted in severe and sometimes fatal shocklike reactions. These reactions, along with the fact that dogs would often be administered product only part of the year during "mosquito season," allowed the reappearance of microfilariae in the blood again in the spring. This was why it became important to test dogs on prevention again before daily heartworm prevention was reinitiated. There was also concern that, after adulticide therapy, dogs might still react because of the dying of their microfilariae when placed back on daily DEC preventive. " . . . . A treated dog cannot be given diethylcarbamazine as a preventive until it is microfilariae free. Some microfilaria-positive dogs that are given diethylcarbamazine will react by going into shock, which is sometimes fatal."[23] Thus, with the introduction of the macrocyclic lactone molecules as proposed heartworm preventives, there was concern that these molecules might also cause similar reactions in dogs with circulating microfilariae.

After the safety work was completed, it was shown that the macrocyclic lactones offered the potential benefit of being microfilaricidal without the frequent, serious (life-threatening) reactions reported with the use of DEC in microfilaremic dogs. In 1980, Powers and coworkers [24] reported on 10 experimentally infected microfilaremic beagles treated with the recommended dose of DEC, and all 10 dogs had adverse reactions, with 7 of the 10 in collapse 1 hour after treatment, and with 1 dog dying of the complications. That same year at the same meeting of the American Heartworm Society, Jackson and Seymour[25] showed that a dose of 250 to 5000 μg ivermectin/kg to dogs with existing infections and microfilaremias did not cause adverse events except in 3 dogs administered very high doses (4 to 48 times the 250 μg/kg dose). However, in 5 of 27 client-owned dogs treated with 250 μg/kg 2 weeks after adulticide therapy with thiacetarsamide, 5 of the dogs reacted and 2 had signs "more severe than transient listlessness and anorexia". All but 1 of these 5 dogs had microfilarial counts over 36,800 per milliliter of blood; 1 dog only had 156 microfilariae per milliliter at the time of treatment and developed listlessness after treatment that lasted 48 hours. Overall, however, even though there were reactions in some microfilaremic dogs treated with ivermectin, when comparing these 2 studies, the potential advantages of using macrocyclic lactones as a microfilaricidal treatment are obvious.

The Center for Veterinary Medicine of the US Food and Drug Administration (CVM/USFDA), in its continuing aim to produce products with high efficacy and safety, has required that the macrocyclic lactones be tested during development in heartworm-positive dogs to monitor the effects of the treatment on dogs with circulating microfilariae. Thus, although all the macrocyclic lactone products on the market have effects on circulating microfilariae, they have been deemed sufficiently safe to warrant release for administration to dogs under the supervision of veterinarians (Table 1). All of the product labels indicate that dogs should be tested for existing heartworm infection before starting treatment.

The macrocyclic lactones used in the United States for heartworm prevention have been shown to be safe for microfilaremic dogs at the prescribed preventive dose. In a multicenter study examining the safety of administering either 2 μg/kg or 10 μg/kg ivermectin orally monthly for a total of 3 doses to dogs with existing heartworm infections, some of the 72 dogs treated had posttreatment reactions including vomiting and excess salivation.[26] In the treatment of random-source microfilaremic and antigen-positive dogs with the prophylactic dose of ivermectin (16 dogs) or milbemycin oxime (12 dogs), adverse reactions at the time of treatment were not reported.[27] Similarly, the treatment of 16 microfilaremic and antigen-positive dogs with the prophylactic dose of ivermectin (8 dogs) or milbemycin oxime (8 dogs) was not accompanied by adverse reactions.[28] Also, there were no reactions to treatment of dogs with circulating microfilariae caused by the transplantation of adult worms 1 month before treatment with prophylactic doses of either ivermectin (5 dogs) or milbemycin oxime (5 dogs).[29] Treating 10 naturally infected (antigen-positive) and microfilaremic dogs with the 3× dose of moxidectin–sustained release formulation did not induce any adverse events.[30] Eight dogs that had received 10

Table 1. Precaution Statements Relative to Heartworm Infection on the Representative Monthly Pioneer* Products

HeartGard Plus, APPROVED 1987; NADA 140-971†: All dogs should be tested for existing heartworm infection before starting treatment with HeartGard Plus, which is not effective against adult *Dirofilaria immitis*. Infected dogs must be treated to remove adult heartworms and microfilariae before initiating a program with HeartGard Plus. Although some microfilariae may be killed by the ivermectin in HeartGard Plus at the recommended dose level, HeartGard Plus is not effective for microfilariae clearance. A mild hypersensitivity-type reaction, presumably due to dead or dying microfilariae and particularly involving a transient diarrhea, has been observed in clinical trials with ivermectin after treatment of some dogs that have circulating microfilariae.

SENTINEL® FLAVOR TABS (INTERCEPTOR), **APPROVED 1995; NADA 141-084‡:** Prior to administration of SENTINEL FLAVOR TABS, dogs should be tested for existing heartworm infections. Infected dogs should be treated to remove adult heartworms and microfilariae prior to initiating treatment with SENTINEL FLAVOR TABS. Mild, transient hypersensitivity reactions manifested as labored respiration, vomiting, salivation and lethargy have been noted in some treated dogs carrying a high number of circulating microfilariae. These reactions are presumably caused by release of protein from dead or dying microfilariae.

Revolution (Pfizer Animal Health, New York, NY), APPROVED 1999; NADA 141-152: Before administration of Revolution, dogs should be tested for existing heartworm infections. At the discretion of the veterinarian, infected dogs should be treated to remove adult heartworms. Revolution is not effective against adult *D. immitis* and, although the number of circulating microfilariae may decrease after treatment, Revolution is not effective for microfilariae clearance. Hypersensitivity reactions have not been observed in dogs with patent heartworm infections administered 3 times the recommended dose of Revolution. Higher doses were not tested.

ProHeart 6, APPROVED 2001, NADA 141-189: Before administration of ProHeart 6, dogs should be tested for existing heartworm infections. Infected dogs should be treated to remove adult heartworms. ProHeart 6 is not effective against adult *D. immitis* and, although the number of circulating microfilariae may decrease after treatment, ProHeart 6 is not effective for microfilariae clearance.

Advantage Multi for Dogs Topical Solution, APPROVED 2006; NADA 141-251: Before administration of Advantage Multi for Dogs, dogs should be tested for existing heartworm infection. At the discretion of the veterinarian, infected dogs should be treated with an adulticide to remove adult heartworms. Advantage Multi for Dogs is not effective against adult *D. immitis*. Although the number of circulating microfilariae may decrease after treatment, Advantage Multi for Dogs is not effective for microfilariae clearance. Safety Study in Heartworm-Positive dogs: Advantage Multi for Dogs was administered topically at 1× and 5× the recommended dose every 14 days for 3 treatments to dogs with adult heartworm infections and circulating microfilariae. At 5×, one dog was observed vomiting 3 hours after the second treatment. Hypersensitivity reactions were not seen in the 5× treatment group. Microfilariae counts decreased with treatment.

Iverhart Max Chewable Tablets (Virbac Corporation, Fort Worth, TX), APPROVED 2006; NADA 141-257: All dogs should be tested for existing heartworm infection before starting treatment with Iverhart Max Chewable Tablets, which are not effective against adult *D. immitis*. Infected dogs should be treated to remove adult heartworms and microfilariae before initiating a heartworm prevention program.

TRIFEXIS™ CHEWABLE TABLETS, APPROVED **2011; NADA 141-321**: Prior to administration of TRIFEXIS, dogs should be tested for existing heartworm infection. At the discretion of the veterinarian, infected dogs should be treated with an adulticide to remove adult heartworms. TRIFEXIS is not effective against adult *D. immitis*. While the number of circulating microfilariae may decrease following treatment, TRIFEXIS is not indicated for microfilariae clearance (see ANIMAL SAFETY). Mild, transient hypersensitivity reactions manifested as labored respiration, vomiting, salivation and lethargy, have been noted in some dogs treated with milbemycin oxime carrying a high number of circulating microfilariae. These reactions are presumably caused by release of protein from dead or dying microfilariae.

***Pioneer indicates original product that generic products copy. The generic product must have the same label insert as the pioneer product in terms of indications, precautions, dosing, etc.**

†Originally, MAR 87, approved as HeartGard 30 (NADA 138-412); then, JUL 89, as HeartGard 30 Chewables (NADA 140-886); and as HeartGard Plus (NADA 141-971) in JAN 93.

‡Originally, DEC 92, approved as Interceptor Flavor Tabs (NADA 141-915); and APR 97, approved as Sentinel Flavor Tabs (NADA 141-084).

pairs of adult worms 7 months previously and that had microfilarial counts over 10,000 per milliliter had no adverse events noted at treatment with selamectin at the preventive rate. However, in a second set of 8 dogs treated monthly beginning 1 month after the worms were transplanted (and for an additional 17 months), there was a single reaction with 1 dog vomiting after the first treatment.[31] Although there may be occasional reactions to all products, varying from mild to severe, these studies clearly demonstrate the overall safety of these products in microfilaremic dogs.

Choosing an Antigen or Microfilarial Test for Dogs on Macrocyclic Lactone Heartworm Prevention

With the widespread use of macrocyclic lactone preventives, it became important to determine the best form of diagnosis for annual testing. The monthly products began to enter the market at about the same time as the antigen tests for heartworm diagnosis. An issue that arose at this time was whether some products, at monthly preventive doses, were microfilaricidal while others were not. The older DEC daily would allow microfilariae to rebound if a dog had an active infection with adult male and female worms,[32] and thus, as stated previously, at the beginning of each "heartworm" season, dogs would be checked annually for circulating microfilariae with one of the many tests. So, the question was which test should be used each spring in dogs on a monthly product? An antigen test or a microfilarial detection test? Toward defining the relationship, several studies showed that both ivermectin and milbemycin oxime administered at the monthly preventive dose suppressed microfilariae in dogs with existing heartworm infections.[27-29] Also, if dogs with heartworms were administered milbemycin oxime for only 6 months, the microfilarial counts were still negative or very low after 6 months without treatment.[27] Thus, the testing modality of choice for the annual heartworm examination changed from microfilarial testing to antigen testing. This has meant that the majority of veterinarians for the last 15 or so years have not been as interested in finding microfilariae as they had been in the past, and that little attention has been given recently to looking for microfilariae in treated or untreated dogs regardless of whether they had received adulticide therapy.

Lack of Microfilarial Clearance in Dogs with Patent Infections Started on Preventive Therapy

In the studies that examined the ability of preventive doses of these drugs to clear microfilariae from the blood of heartworm-infected dogs with microfilariae in circulation, most but not all dogs took at least several months to become amicrofilaremic after beginning monthly heartworm preventive therapy with either ivermectin or milbemycin oxime, and some were never successfully cleared.[33] In one trial, less than half the dogs were positive 6 months after beginning treatment (3 of 6 dogs receiving ivermectin and 5 of 12 dogs receiving milbemycin oxime) and some dogs were positive in each group at the end of the trial (10 or 11 months of monthly treatment).[27] This persistence of microfilariae in the presence of preventive doses of macrocyclic lactones is also the case with selamectin and moxidectin as ProHeart 6.[30,31] For moxidectin as Advantage Multi for Dogs Topical Solution at 1× and 5× the monthly dose for 3 treatments 2 weeks apart, microfilariae were not cleared from all dogs (Advantage Multi Label). Some 10% to 20% of heartworm-infected dogs that begin monthly heartworm preventive therapy without adulticide therapy will have the continued and persistent presence of circulating microfilariae.[33] Even higher doses of ivermectin will not necessarily clear circulating microfilariae from the blood of infected dogs. Five heartworm-infected dogs treated with 250 μg of ivermectin/kg (2 consecutive days, 2 consecutive days 6 days later, and 1 dose 4 days thereafter) failed to clear circulating microfilariae from 4 of the dogs with the exception being 1 dog that cleared after only the first 4 doses.[25]

Recrudescence is a term that refers to the reappearance of microfilariae in the blood of heartworm-infected dogs some time after chemical suppression of microfilariae has been withdrawn. In these studies, the majority of the dogs have remained heartworm antigen positive, indicating the presence of adult worms.[34] In 6 dogs administered milbemycin oxime for 6 months, 1 dog fully recrudesced 5 months after the last treatment, and another of the 6 dogs had a few microfilariae in circulation 8 months after the last treatment.[27] In 10 dogs that had received either 9 monthly preventive doses of ivermectin (5 dogs) or milbemycin oxime (5 dogs), 4 of the 5 dogs in both groups were cleared of their microfilariae.[28] Two of the milbemycin oxime–treated dogs and 1 of the ivermectin-treated dogs recrudesced 4 and 6 months, respectively, after the termination of treatment. Because of the decrease in circulating microfilariae that occurs after macrocyclic lactone therapy, antigen testing is the best means of detecting an infection in a dog that has some history of treatment with macrocyclic lactones. However, microfilariae are also liable to be present in the circulation of dogs that have been withdrawn from macrocyclic lactone therapy for some time.

The appearance or timing of the appearance of microfilariae in the blood of dogs that are infected before they begin prophylaxis is determined by the time of first treatment relative to the time of infection. If dogs receive monthly therapy with macrocyclic lactones around 3 to 3.5 months after infection, it will suppress microfilaremias even if adult worms develop in the animals.[35,36] Similarly, the administration of moxidectin in the sustained-release formulation of ProHeart 6 at either 4 or 6 months after infection will suppress microfilaremias in dogs that develop adult worms.[37] However, in those cases in which older infections have been treated with monthly preventive formulations of either milbemycin oxime or ivermectin, transient microfilaremias develop. When started at 4.5 months after infection, 86% of the dogs developed patent infections (albeit at low numbers and only for

about 3 months), and when started at 5.5 or 6.5 months after infection, 100% of the dogs had patent infections for 3 or 4 months beginning around 190 days after infection.[35] In these dogs, the microfilaria counts were slightly higher in the ivermectin dogs when compared with the milbemycin oxime–treated dogs. In a similar study of dogs that started monthly ivermectin/pyrantel pamoate at 5 and 7 months after infection, only 2 of the dogs starting therapy at 5 months developed patent infections: 1 dog had 2 to 31 microfilariae per milliliter when sampled at 7 to 9 months after infection and another had 3 to 18 microfilariae per milliliter when sampled at 8 and 9 months after infection.[36] All the dogs that started preventive therapy at 7 months developed patent infections with a peak of 3812 microfilariae per milliliter at 9 months after infection, which then decreased, with none being seen in the peripheral blood after 13 months after infection. Thus, if dogs are treated for the first time with the preventive 3 or 4 months after infection, the chance of developing patent infections is minimal, but every week thereafter, it appears that the chance of dogs having circulating microfilariae increases.

The concern with the extended presence of microfilariae in dogs on a long-term preventive regimen has been of concern because these microfilariae are persisting in the presence of the macrocyclic lactones.[38] The worry is that these microfilariae have been selected for resistance to these molecules, and the mosquitoes have the ability to transfer worms that have already been selected for their ability to survive in the presence of these products. The results of ongoing research with heartworm isolates from LOEs in the Mississippi Delta Region supports further discussion on this topic.

Macrocyclic Lactones as Adjuncts to Adulticide Therapy

The American Heartworm Society recommends that macrocyclic lactones be used to treat all dogs for up to 3 months before they are treated with an adulticide.[39] The purpose of this treatment is to allow worms that are less than 7 months old to reach full maturity while preventing any new worms from mosquitoes taking up residence in the dog. ". . . It is beneficial to administer a macrocyclic lactone for up to three months before administration of melarsomine, when the clinical presentation does not demand immediate intervention. The logic for this approach is to kill susceptible heartworm larvae and thus prevent re-infection of the dog, while allowing less susceptible juvenile worms, the opportunity to develop into more susceptible adult worms. This tactic increases the chance for removal of the existing heartworm infection when the adulticide injections are given later. Additional benefits of this protocol are the effects of macrocyclic lactones in greatly reducing, if not eliminating circulating microfilariae, stunting immature *D.Dirofilaria immitis* and reducing female worm mass by compromising the reproductive system." As stated by the American Heartworm Society: "While controversial due to the theoretical risk of selecting heartworm populations that are resistant to macrocyclic lactones, it is beneficial. . ."; Although the risk is minimal that any single dog would transmit infection of microfilariae living in the presence of a macrocyclic lactone, at the same time it must be remembered that tens of thousands of dogs are treated for heartworms annually in the United States, and this practice may increase that risk. However, whether dogs are started on several months of therapy before killing the adult worms, or started on a macrocyclic lactone preventive at the time of adulticide therapy or immediately before or after adulticide treatment (i.e., without delay), it is warranted to prevent additional infection of the dog being treated.

Macrocyclic Lactones as Microfilaricides after Adulticide Therapy

There is currently no FDA-approved microfilaricide for use after adulticide treatment available in the United States. Although none of the macrocyclic lactones are approved currently as microfilaricides by the Center for Veterinary Medicine, under the Animal Medicinal Drug Use Clarification Act of 1994, licensed veterinarians are permitted extra-label uses of certain drugs that have an established clinical application, if a valid veterinarian-client-patient relationship exists (http://www.fda.gov/AnimalVeterinary/default.htm). The widespread use of these macrocyclic lactone chemoprophylactics as microfilaricides is governed by this regulation. The documents summarizing the preclinical and clinical trials with these products appear on the FDA's web site (www.fda.gov) and supply additional data on the safety of these products in microfilaremic dogs.

The microfilaricidal effects of macrocyclic lactones were first examined before the availability of melarsomine dihydrochloride when the treatment that was administered was intravenous thiacetarsamide. Initially, an experiment showed that after thiacetarsamide therapy, all dogs tested could be cleared of their circulating microfilariae with a single dose of 250 μg ivermectin/kg.[25] Further work showed that out of 121 dogs treated with thiacetarsamide and then treated 2 weeks later with 50 μg ivermectin/kg, only 5 dogs failed to be cleared of microfilariae with 1 dose; it was believed that these dogs were ultimately cleared of their microfilariae because the adult worms were gone.[40] In another series using dogs treated with 200 μg/kg of ivermectin subcutaneously 2 weeks after thiacetarsamide therapy, out of 62 dogs, all were negative 21 days after ivermectin treatment.[41] However, 4 dogs had microfilariae reappear day 42 after ivermectin treatment, and 3 of these 4 dogs still had worms at necropsy (out of the 11 control and treated dogs in this part of the study, 4 control dogs and 6 treated dogs had worms in the pulmonary vasculature at necropsy). Milbemycin oxime was used as a microfilaricidal in a study 2 weeks after thiacetarsamide therapy in 40 dogs with naturally acquired heartworms.[42] There were 8 control dogs, and 32 dogs (8 per group) treated with 0.01, 0.10, 0.25, and 0.50 mg milbemycin oxime/kg, and microfilarial counts were performed before treatment and on days 1, 3, and 7, and then weekly after treatment. By the end of the study 42 days after treatment, only 7 treated dogs had blood clear of microfilariae (3 in the 0.10 mg/kg, 1 in the 0.25

mg/kg, and 3 in the 0.50 mg/kg groups). Thus, clearance only occurred in 21.9% of the treated dogs and in only 37.5% of the dogs that received the current preventative dose of 0.50 mg milbemycin oxime/kg. Based on necropsies, only about half of the dogs in each treatment group had been cleared of their infections. Again, in these studies in which perhaps not all dogs were cleared of their adult heartworms, clearance of microfilariae may take months or never occur.

There have been surprisingly few published reports on the actual clearance of microfilariae after treatments with melarsomine dihydrochloride (Immiticide/RM340, Immiticide Sterile Powder NADA 141-042). In the treatment of 44 dogs with melarsomine, 33 of 44 were positive for microfilariae at the time of melarsomine treatment, and 4 months later, after most of the dogs had received postadulticide therapy and started prophylaxis with either ivermectin or milbemycin oxime, only 1 of the dogs was microfilarial positive.[43] In Germany, 19 dogs naturally infected with *Dirofilaria immitis* were treated with an intramuscular injection of 2.5 mg melarsomine dihydrochloride/kg at an interval of 24 hours, and 6 weeks later with 0.1 mg ivermectin subcutaneously/kg.[44] All 19 dogs were negative for microfilariae 3 weeks after ivermectin treatment, although 2 dogs remained antigen enzyme-linked immunosorbent assay positive. This is the same group of dogs mentioned earlier, in which 2 dogs were still antigen positive, although at a lower level than before treatment. We have very little baseline information on the clearance of microfilariae after adulticide therapy with melarsomine. Given that people are currently concerned about dogs remaining microfilarial positive after becoming antigen negative after adulticide treatment despite being treated repeatedly with ivermectin at 200 μg/kg, it is unfortunate that we have not collected more data on microfilarial persistence in dogs that became antigen negative after melarsomine.

Adverse Events Associated with Macrocyclic Lactones as Microfilaricides after Adulticide Therapy

There have also been very few studies on the adverse events that occur when these products have been used as microfilaricides after heartworm preventive therapy. In 1983, Plue and coworkers[41] reported on the treatment of 31 random-source, mixed-breed dogs with 200 μg/kg of ivermectin subcutaneously 2 weeks after adulticide therapy with thiacetarsamide. In the first 2 trials (10 treated dogs each), there were no reactions. In the third trial with 11 treated dogs, 8 dogs reacted: one in as few as 12 minutes after treatment. The authors felt that the reactions were not due to the rate of microfilarial clearance because some dogs with counts of 50,000 microfilariae per milliliter were cleared by 24 hours after treatment without reacting. Milbemycin oxime was used in 32 dogs 2 weeks after thiacetarsamide treatment; each group of 8 dogs received either 0.01, 0.1, 0.25, or 0.5 mg milbemycin oxime/kg (the latter is the routine monthly preventive dose) 1 time.[42] There were 4 reported mild reactions, one 0.5 mg/kg treated dog was depressed on day 0, one of the 0.25 mg/kg dogs vomited within 6 hours of treatment, one 0.25 mg/kg dog had excessive salivation noted 2 days after the microfilaricidal treatment, and one dog receiving 0.01 had coughing and slight depression 2 weeks after treatment (this dog still harbored 44 worms at necropsy 56 days after thiacetarsamide treatment). Only half (16 of the 32 treated dogs) were cleared of their adult heartworms, and only 2 of these dogs were negative for microfilariae 42 days after milbemycin treatment (one in the 0.1 mg/kg group and one in the 0.5 mg/kg group). In Germany, 20 dogs naturally infected with *Dirofilaria immitis* were treated with melarsomine dihydrochloride in the 2-injection regimen (intramuscularly at 2.5 mg/kg at an interval of 24 hours), and then, 6 weeks later, they were treated with ivermectin (subcutaneously at 0.1 mg/kg).[44] No adverse events were reported. Thus, it would appear that macrocyclic lactones, especially, compared with DEC as stated earlier are safe to administer to microfilaremic dogs after adulticide therapy.

Effects of the Macrocyclic Lactones on the Microfilariae

The effects of macrocyclic lactones on the numbers of circulating microfilariae may be due to several different underlying causes. There is probably some direct effect on the nervous system of microfilariae that causes them to lose motility and viability.[2] It is also known that the repeated application of the product changes the predominance of stages found in the uterus of the female worms.[34] Usually, the uterus of a female heartworm contains a succession that goes from the vulva toward the oviduct of stretched microfilariae, pretzel-folded microfilariae (still in the eggshell), developing embryos in eggshells, and the prelarval morula stages. With repeated treatment by macrocyclic lactones, the only stage that remains after some period of time is the prelarval stage, i.e., the eggs do not undergo development to microfilariae within the uterus. One other possibility that has been investigated is potential effects on the sperm within male worms. This was shown to be a possibility by transplanting drug-sterilized female worms along with untreated normal males into dogs and showing that the females recovered their ability to produce larvae, which they did not do if they were transferred along with treated males.[34] Thus, although we know some mechanisms, the details of all mechanisms by which microfilariae are killed and cleared in dogs treated with macrocyclic lactones have not yet been fully elucidated.

Concern That Repeated Treatment of Dogs with Patent Infections May Predispose Heartworm Infections toward Selection of Macrocyclic-resistant Forms

The work discussed previously, in which macrocyclic lactones administered repeatedly to dogs with patent infections routinely failed to clear some percentage of dogs of their

circulating microfilariae even after 10 to 12 months of treatment, suggests that these dogs could be a source of macrocyclic lactone–resistant microfilariae (Fig 3). Mosquitoes could then transmit these resistant forms to other dogs. This could theoretically lead to a population of worms being transferred between dogs that have increased resistance to this class of molecule. The increased numbers of LOEs that were reported from the Mississippi Delta Region have been suggested by some to be due to resistance to this class of preventive products. Using an in vitro microfilaricidal assay, Blagburn and coworkers showed that microfilariae recovered from dogs undergoing LOE events while on different macrocyclic lactone preventives in the lower Mississippi Delta Region required higher concentrations of macrocyclic lactones than baseline-susceptible dogs to reach a 95% lethal dose (LD95).[2] The effect has been observed with all 4 of the commercially available macrocyclic lactones that have been tested in their chemical-grade formulations (Blagburn AHS 2010 meeting abstract, and personal communication). The microfilaria isolates from the field with higher LD95s have maintained their high LD95s in the F1 generation in laboratory-infected dogs. It has also been shown molecularly that the P-glycoprotein genes of the forms that phenotypically display the LOE genotype have undergone increased homozygosity for this gene sequence compared with microfilariae from heartworms that have LOEs equivalent to the fully macrocyclic lactone–susceptible isolates maintained in the laboratory.[45,46] The tendency toward homozygosity for this gene is a marker for macrocyclic lactone resistance in trichostrongyloid nematodes of ruminants and has been suggested to be a marker for macrocyclic lactone resistance in the related filarioid *Onchocerca volvulus*.[47] Thus, although it awaits confirmatory trials in which worms are shown to develop successfully in some number in dogs receiving monthly (or 6-month injectable) preventive therapy, the indications are present that we might be dealing with heartworm resistance to macrocyclic lactones in at least some areas of the country.

Doxycycline, *Wolbachia*, and Microfilariae

Wolbachia is an alphaproteobacterial endosymbiont of insects and other arthropods that has recently been under investigation relative to the treatment and prevention of heartworm infections. There are many nematodes that contain these organisms, those that do typically have an arthropod intermediate host in their life cycle, and thus it is believed that the nematode has become infected with the insect's endosymbiont. *Wolbachia* was originally noted in filarioids and heartworm back in 1975 when the electron microscope was first directed to the examination of filarial parasites.[48] It was discussed as a potential target of chemotherapy for human filariasis in which DEC was used routinely, and then the idea was supplanted in the 1980s with the discovery of the wonder-drug ivermectin. The bacteria in *Dirofilaria immitis* was fairly well forgotten until 1995, when it was redefined as a *Wolbachia* using molecular methods,[49] and its potential importance to human filarial diseases was later recognized.[50] Tetracycline was shown to have some efficacy against *Wolbachia* in the human parasite *Brugia pahangi* and the canine *D. immitis*.[51]

Then, in a cattle filarioid model for human onchocerciasis (*Onchocerca ochengi*), it was shown that long-term tetracycline therapy could have the effect of destroying the parasites within their subcutaneous nodules.[52] It was hoped that the effects of treatments targeting the *Wolbachia* organisms would have similar effects on the human parasite *O. volvulus*. The infection is passed from worm to worm through the egg, and the infection is carried from host to host in the microfilaria to the mosquito and in the third-stage larva back into the host. The organisms are present in the male, but it is only through the female that the organisms are passed onto the next generation.

Unfortunately, the effects of killing *Dirofilaria immitis* and the human pathogen *Onchocerca volvulus* by destroying the bacterial endosymbiont have not worked out quite as well as first hoped based on the success obtained with *O. ochengi*. There are effects on the worm, but it appears that the dog heartworm is not sufficiently dependent on its bacterial symbiont to be killed with simple prolonged antibiotic (doxycycline) therapy alone (see below). However, preliminary evidence has indicated that the clearing of the *Wolbachia* from the microfilarial stage in the blood of the dog might serve to prevent the infective larvae that develop in the mosquito from being able to continue their development in the dog; this would mean that doxycycline may have a role to play in keeping other dogs from being infected even if the dog itself still has its heartworms.[33]

Doxycycline seems to have some minimal effect by itself against microfilarial numbers. The studies looking at the treatment of dogs that are microfilarial and antigen positive have typically used some combination of doxycycline and ivermectin. The regimens included 30-day or long-term intermittent doxycycline therapy with or without ivermectin being administered every week or every other week, plus or minus melarsomine treatment. In a 36-week study, 6 groups of 5 dogs each with transplanted adult worms were treated with weekly ivermectin beginning 6 weeks after infection; intermittent doxycycline (20 of 36 weeks); ivermectin and doxycycline; ivermectin, doxycycline, and melarsomine; or melarsomine alone. The microfilaria were gone from the dogs treated with ivermectin and doxycycline (with and without melarsomine) after the ninth week of infection, but remained persistent in the dogs that received ivermectin or doxycycline alone throughout the study.[53] In another 34-week study, 20 beagles were infected by the transplantation of worms and were divided into 4 groups 6 weeks later, with 1 getting weekly ivermectin; 1 receiving intermittent doxycycline (24 of 34 weeks); and 1 receiving ivermectin and doxycycline. There was also a group of untreated controls. Although microfilarial counts were significantly decreased in all treatment groups versus control dogs from week 12 onward, only the ivermectin and doxycycline group cleared the microfilaria infection by week 12 with a significant reduction in microfi-

laria count from as early as week 6 compared with the control group. Only 2 of the 5 dogs treated with ivermectin weekly were amicrofilaremic.[54] In naturally infected client-owned dogs in Italy treated with doxycycline and ivermectin every 2 weeks for 6 months, the microfilariae were gone from all but 1 dog 8 weeks after infection and from all dogs by 12 weeks after infection.[55] Thus, it would seem that doxycycline alone has some microfilaricidal properties, but its use as a standalone agent for microfilarial clearance may not be warranted.

Antigen and Microfilarial Testing in Dogs Relative to Macrocyclic Lactones Today

The best way to examine the blood of a dog on preventive therapy to determine if there have been compliance issues or a lack of product efficacy is to use an antigen test and a microfilarial detection test. There are occasional dogs that are microfilarial positive and antigen negative, sometimes with very high numbers of microfilariae. The reason behind this is not clear, but it does occur. On initial evaluation for heartworm infection in dogs ≥ 6 months of age with an unknown history of exposure and/or heartworm prevention, both tests are indicated. If nothing else, this could prevent confusion down the road. However, for the annual test, the antigen test is still the test of first choice.

It is becoming fairly common again for people to check the blood of dogs that have been treated for heartworms to verify postadulticide microfilarial clearance with some form of macrocyclic lactone treatment. It is unclear what is underlying this renewal in microfilarial checking, but it is probably a valid medical approach. After adulticide treatment and microfilaricidal therapy, dogs should be verified to be clear of circulating microfilariae so that they do not remain a potential source of infection for other dogs. Concern remains that the administration of product to dogs that have persistent circulating microfilariae will serve as a means of presenting mosquitoes with a population of microfilariae that have been preselected for macrocyclic lactone resistance by surviving the regular killing of the susceptible microfilariae with which they are sharing the dog. Thus, if there is a potential that resistance is occurring, it may be more important than previously to verify after adulticide therapy that dogs have stopped being a threat to their neighbor.

Conclusion

Macrocyclic lactones remain our only class of heartworm prevention available, and preserving their effectiveness is critical. Heartworm prevention in the canine host has focused on killing the third-stage larvae from the biting mosquito and the early fourth-stage larvae into which they develop, but macrocyclic lactones also have microfilaricidal properties. The microfilaricidal properties of all these products, along with the already well-known presence of occult infections, have changed heartworm diagnostics such that the routine annual checks for circulating microfilariae have been replaced by antigen detection tests. Testing for microfilariae still has value: because a few dogs can be microfilaria positive and antigen negative, it can be advantageous to test all dogs >7 months of age for both antigen and microfilariae before beginning preventive therapy. After adulticide treatment and microfilaricidal therapy, dogs should be verified to be clear of circulating microfilariae so that they do not remain a potential source of infection for other dogs.

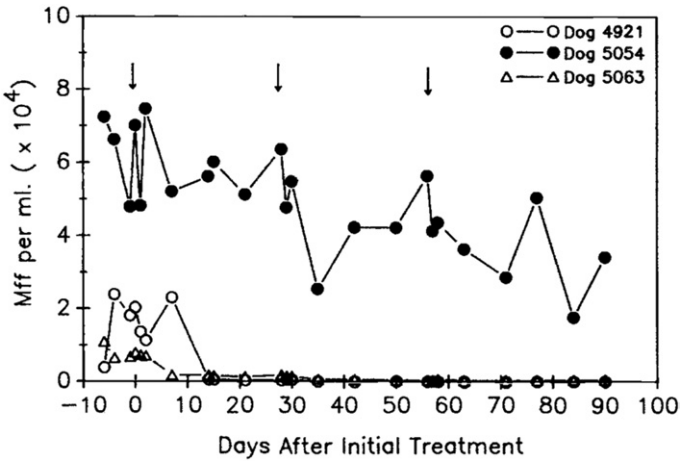

Figure 3. Effects of 3 monthly treatments with oral ivermectin preventive on the numbers of microfilariae per milliliter in 3 naturally infected dogs. Downward-pointing arrows indicate time of oral treatment with tablets of HeartGard (treated at 6-12 μg/kg once each month). Reprinted with permission.[34]

Macrocyclic lactones offer the benefit of being microfilaricidal without the frequent, serious (life-threatening) reactions reported with the use of DEC in microfilaremic dogs. Although there may be occasional reactions to all products, varying from mild to severe, studies clearly demonstrate the overall safety of these products in microfilaremic dogs. All of the product labels indicate that dogs should be tested for existing heartworm infection before starting treatment. Microfilarial clearance may take months, and may never occur in some dogs with adult infections, despite receiving heartworm preventives. Although we understand some of the effects of macrocyclic lactones on circulating microfilariae (direct effect on the nervous system, changing the predominance of stages found in the uterus of the female worms), we do not know complete details of all mechanism by which microfilariae are killed and/or cleared in dogs treated with macrocyclic lactones. If dogs are treated for the first time with the preventive 3 or 4 months after infection, the chance of developing patent infections is minimal, but every week thereafter the chance of dogs having circulating microfilariae increases. Persistent circulating microfilariae will serve as a means of presenting mosquitoes with a population of microfilariae that have been preselected for macrocyclic lactone resistance by surviving the regular killing of the susceptible microfilariae with which they are sharing the dog.

In the dog, at very least, *Dirofilaria immitis* does not appear sufficiently dependent on its bacterial symbiont *Wolbachia* to be killed with simple prolonged antibiotic (doxycycline) therapy alone, but clearing of the *Wolbachia* from the microfilarial stage in the blood of the dog might serve to prevent the infective larvae that develop in the mosquito from being able to continue their development in the dog. This is a new avenue of research that is being pursued in several laboratories around the world.

We have been very fortunate to have these products for heartworm prevention in dogs and we need to constantly reassess where we stand relative to how they are used to prevent infections in dogs such that we can extend the usefulness of this class of molecules for as long as possible.

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