Current Canine Guidelines for the

Prevention, Diagnosis, and Management of Heartworm *(Dirofilaria immitis)* Infection in Dogs

AMERICAN HEARTWORM SOCIETY

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(revised July 2014)

CONTENTS

Click on the links below to navigate to each section.

Preamble

These recommendations supersede previous editions and are based on the latest information presented at the 2013 Triennial Symposium of the American Heartworm Society (AHS), new research, and additional clinical experience. The recommendations for the prevention, diagnosis, and management of heartworm infection in cats are contained in a companion feline document (http://heartwormsociety.org/veterinary-resources/feline-guidelines.html).

HIGHLIGHTS

- **Diagnostics:** AHS recommends annual antigen and microfilaria testing. (Because the interpretation of diagnostics has become more complex, please see the "Microfilaria and Antigen Testing" section for more complete information.)

- **Chemoprophylaxis:** AHS recommends year-round administration of chemoprophylactic drugs to prevent heartworm disease, control other pathogenic and/or zoonotic parasites, and enhance compliance, the latter being particularly important in light of the documented presence of resistant subpopulations.

- **Adulticide therapy:** AHS recommends use of doxycycline and a macrocyclic lactone prior to the three-dose regimen of melarsomine (one injection of 2.5 mg/kg body weight followed at least one month later by two injections of the same dose 24 hours apart) for treatment of heartworm disease in both symptomatic and asymptomatic dogs.

Any method utilizing only macrocyclic lactones as a slow-kill adulticide is not recommended.

EPIDEMIOLOGY

Heartworm infection in dogs has been diagnosed around the globe, including all 50 of the United States. In the United States, its territories, and protectorates, heartworm is considered at least regionally endemic in each of the contiguous 48 states, Hawaii, Puerto Rico, U.S. Virgin Islands, and Guam (Bowman et al, 2009; Kozek et al, 1995; Ludlam et al, 1970). Heartworm transmission has not been documented in Alaska; however, there are regions in central Alaska that have mosquito vectors and climate conditions to support the transmission of heartworms for brief periods (Darsie and Ward, 2005; Slocombe et al, 1995; Terrell, 1998). Thus, the introduction of microfilaremic dogs or wild canids could set up a nidus of infection for local transmission of heartworm in this state. Such relocation of microfilaremic dogs and expansion of the territories of microfilaremic wild canids in other areas of the United States continue to be important factors contributing to further dissemination of the parasite, as the ubiquitous presence of one or more species of vector-competent mosquitoes makes transmission possible wherever a reservoir of infection and favorable climatic conditions co-exist. Change in any of these factors can have a significant effect on the transmission potential in a specific geographic location.

Environmental changes, both natural climatic change and those created by humans, and animal movement have increased heartworm infection potential. Commercial and residential real estate development of non-endemic areas and areas of low incidence has led to the resultant spread and increased prevalence of heartworms by altering drainage of undeveloped land and by providing water sources in new urban homesites. In the western United States, irrigation and planting of trees has expanded the habitat for *Aedes sierrensis* (western knot hole mosquito), the primary vector for transmission of heartworms in those states (Scoles et al, 1993).

Aedes albopictus (Asian tiger mosquito), which was introduced into the Port of Houston in 1985, has now spread northward, approaching Canada, and isolated populations have been identified in areas in the western states (Scoles and Dickson, 1995). This urban-dwelling mosquito is able to reproduce in small containers, such as flowerpots
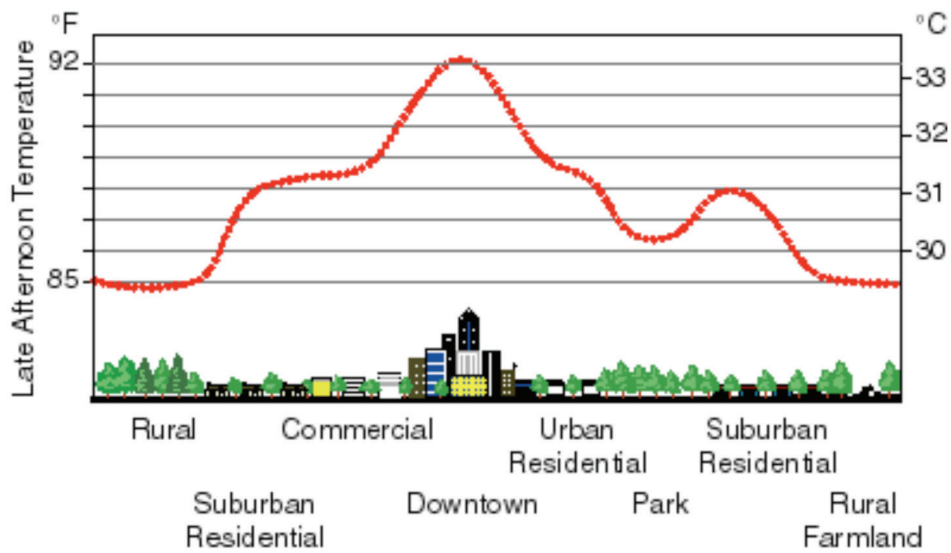
Figure 1. Sketch of an urban heat island profile. From http://eetd.lbl.gov/HeatIsland/HighTemps/

(Benedict et al, 2007). Urban sprawl has led to the formation of "heat islands," as buildings and parking lots retain heat during the day (Figure 1), creating microenvironments with potential to support the development of heartworm larvae in mosquito vectors during colder months, thereby lengthening the transmission season (Morchón et al, 2012).

As vectors expand their territory, the number of animals infected will continue to increase. A pivotal prerequisite for heartworm transmission is a climate that provides adequate temperature and humidity to support a viable mosquito population, and also sustain sufficient heat to allow maturation of ingested microfilariae into the infective, third-stage larvae (L3) within this intermediate host. It has been shown that maturation of larvae, within three mosquito species, ceases at temperatures below 57°F (14°C) (Christensen and Hollander, 1978; Fortin and Slocombe, 1981). Heartworm transmission does decrease in winter months, but the presence of microenvironments in urban areas suggests that the risk of heartworm transmission never reaches zero. Furthermore, some species of mosquitoes overwinter as adults. While heartworm larval development in these mosquitoes may cease in cool temperatures, development quickly resumes with subsequent warming (Ernst and Slocombe, 1983).

The length of the heartworm transmission season in the temperate latitudes is critically dependent on the accumulation of sufficient heat to incubate larvae to the infective stage in the mosquito (Knight and Lok, 1998; Lok and Knight, 1998). The peak months for heartworm transmission in the Northern Hemisphere are typically July and August. Models predict that

heartworm transmission in the continental United States is limited to 6 months or less above the 37th parallel at approximately the Virginia–North Carolina state line (Guerrero et al, 2004). While model-based predictions of transmission using climatic data are academically appealing, they typically fail to consider several potentially important factors, such as influence of microclimate, unique biological habits and adaptations of the mosquito vectors, variations in time of larval development, mosquito life expectancy, and temperature fluctuations. Predictive risk maps assume that mosquito vectors live for only one month; however, several significant mosquito vectors live and breed for much longer periods, including *Aedes albopictus* (3 months) (Löwenberg Neto and Navarro-Silva, 2004), *Aedes sticticus* (3 months) (Gjullin et al, 1950), *Ochlerotatus (*formerly *Aedes) trivittatus* (2 months) (Christensen and Rowley, 1978), *Aedes vexans* (2 months) (Gjullin et al, 1950), and *Ochlerotatus (*formerly *Aedes) canadensis* (several months) (Pratt and Moore, 1960). There are also documented cases of hibernating *Anopheles quadrimaculatus* surviving for 4 to 5 months (Hinman and Hurlbut, 1940), so the predictive risk maps likely reflect a shorter transmission season than actually exists.

Survey studies of trapped mosquitoes randomly collected at various locations have demonstrated heartworm infection rates in mosquitoes ranging from 2% to 19.4% in known endemic areas. When mosquito sampling was restricted to kennel structures where known positive dogs were being housed, the infection rates of the mosquitoes in these restricted samplings resulted in rates of 30%

American Heartworm Society

adjacent to and 74% inside the facilities (McKay et al, 2013). Based upon these data, it is important to protect pets from mosquito exposure. This may be accomplished by environmental control measures, including treatment of standing water sources with insect growth regulators (IGRs) combined with mosquito adulticidal measures (sprays, CO_2 traps, etc). In addition to mosquito control, keeping pets inside during peak mosquito hours and/or the use of mosquito repellants on pets may also reduce the risk of infection.

Once a reservoir of microfilaremic domestic and wild canids is established beyond the reach of veterinary care, the ubiquitous presence of one or more species of vector-competent mosquitoes makes transmission possible and eradication becomes improbable.

BIOLOGY AND LIFE CYCLE

The domestic dog and some wild canids are the normal definitive hosts for heartworms and thus serve as the main reservoir of infection. Even less suitable hosts, such as cats and ferrets, occasionally

have low-level, transient microfilaremia and therefore, theoretically, may serve as a limited source of infection for mosquitoes during these short periods of microfilaremia (McCall et al, 2008b).

The life cycle of *Dirofilaria immitis* is relatively long (usually 7 to 9 months) compared with most parasitic nematodes (Figure 2) (Kotani and Powers, 1982). The susceptible mosquito becomes infected when taking a blood meal from a microfilaremic host. Microfilariae cannot develop into adult heartworms without first developing into larval stage 1 (L1) in the malpighian tubules of the mosquito, then molting into larval stage 2 (L2), and finally molting into an infective third-stage larva (L3) (Taylor, 1960). The third-stage larvae then migrate via the body cavity to the head and mouthparts of the mosquito, where they become infective. The time required for the development of microfilariae to the infective stage in the mosquito is temperature dependent. At 27°C and 80% relative humidity, development takes about 10 to 14 days; with cooler temperatures maturation takes longer (Kartman, 1953; Slocombe et al, 1989).
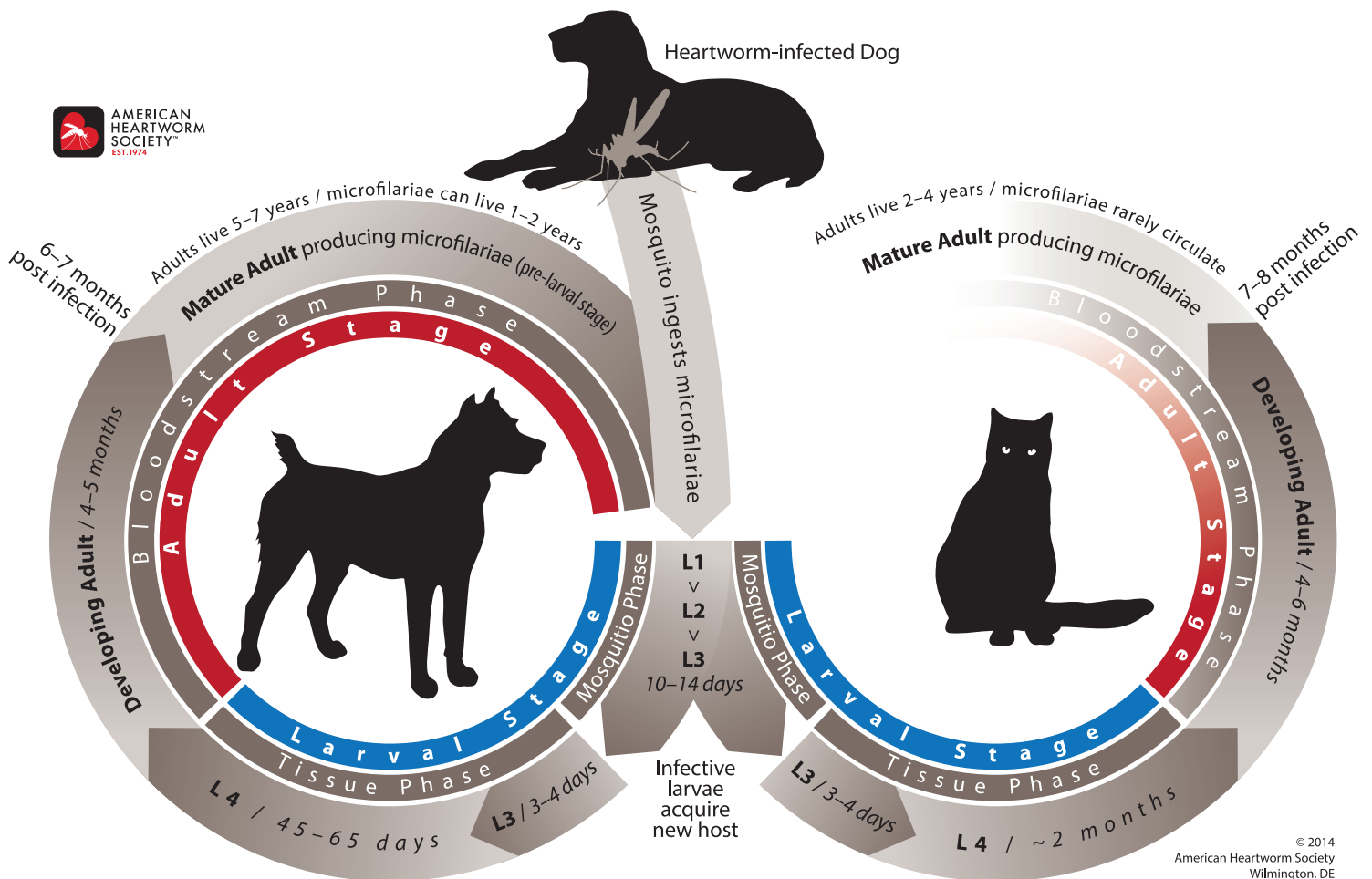

Figure 2. The heartworm life cycle.

When the mosquito takes a blood meal, the infective larvae rupture the end of the mosquito's labrum and emerge within a droplet of hemolymph (mosquito blood) on the skin of the host (McGreevy et al, 1974). Immediately after the blood meal, these sexually differentiated larvae enter the animal's body via the puncture wound made by the mosquito's mouthparts. Apparently L3 and L4 travel between muscle fibers during migration, whereas juveniles (immature adults) penetrate muscle and eventually veins, transporting them toward the heart and lungs (Kotani and Powers, 1982; Kume and Itagaki, 1955; Lichtenfels et al, 1985). The molt from L3 to L4 begins as early as day 3 and ends as late as days 9 to 12. L4 molt to the final stage at days 50 to 70. Immature adult (fifth stage) worms reach the pulmonary vasculature as early as day 67 and all have arrived by days 90 to 120. The first worms entering the pulmonary vasculature on days 67 to 85 are 1 to 1.5 inches in length. Thereafter, adult worms increase in length, with females increasing by almost tenfold, becoming sexually mature about day 120 post infection. Dogs develop patent infections (ie, have circulating microfilariae) as early as 6 months but usually by 7 to 9 months after infection (Kotani and Powers, 1982; Orihel, 1961).

When juvenile heartworms first reach the lungs, the flow of blood forces them into the small pulmonary arteries (Rawlings, 1980). As the worms grow and increase in size, they progressively occupy larger and larger arteries until they become fully mature. The eventual location of the mature adult worms appears to depend mainly on the size of the dog and the worm burden. A medium-sized dog (eg, Beagle) with a low worm burden (ie, ≤5) usually has worms mainly in the lobar arteries and main pulmonary artery. As the worm burden increases, worms also can be located in the right ventricle. Dogs with more than 40 worms are more likely to have caval syndrome, where the worms maneuver into the right ventricle, right atrium, and the vena cavae, thus interfering with valvular function and/or blood flow and producing hemolysis, liver and kidney dysfunction, and heart failure (Atwell and Buoro, 1988; Ishihara et al, 1978; Jackson, 1975).

A clear understanding of heartworm transmission, development, prepatent period, and the susceptibility of the different life stages of the parasite to available pharmaceutical drugs is critical. This knowledge base is necessary to effectively select the most appropriate adulticidal treatment option and treatment time, and to develop realistic expectations for the veterinarian and client for the outcome of therapy.

HEARTWORM PREVENTION

The prescription of heartworm chemoprophylactic medication requires authorization by a licensed veterinarian having a valid relationship with the client and patient. To establish this relationship, heartworm prevention should be discussed with the client. If records of past treatment and testing do not exist, it is necessary to test the patient before dispensing or prescribing chemoprophylaxis. Options for effective chemoprophylaxis include several drugs administered monthly either orally or topically, or parenterally at 6-month intervals.

Heartworm infection is preventable despite the dog's inherently high susceptibility. Because all dogs living in heartworm-endemic areas are at risk, chemoprophylaxis is a high priority. Puppies should be started on chemoprophylaxis as early as possible, no later than 8 weeks of age. Puppies started on a heartworm preventive after 8 weeks of age, or housed unprotected outdoors in heavily endemic areas, should be tested 6 months after the initial dose and annually thereafter. Before initiating a preventive regimen in older dogs (7 months of age or older), antigen and microfilaria testing should be performed (see PRIMARY DIAGNOSTIC SCREENING). This practice avoids delays in detecting subclinical infections and the potential confusion concerning effectiveness of the prevention program if a preexisting infection becomes evident after beginning chemoprophylaxis (eg, chemoprophylaxis initiated during the prepatent period).

Evidence strongly suggests that by reducing the reservoir population through increasing the number of dogs receiving chemoprophylaxis, a disproportionately large decrease in the prevalence of infection among unprotected dogs may occur (Theis et al, 1998). This "collateral" protection spreads the umbrella of chemoprophylaxis most effectively in communities where heartworm prevalence and dog population density are both relatively low.

Even though continuous, year-round transmission may not occur throughout the country, the administration of broad-spectrum chemoprophylactic products with endoparasitic and/or ectoparasitic activity for 12 months each year likely enhances compliance and may assist in preventing pathogenic and/or zoonotic parasitic infections.

Macrocyclic Lactones

The heartworm preventives currently marketed (ivermectin, milbemycin oxime, moxidectin, and selamectin) belong to the macrocyclic lactone class of drugs. These drugs affect microfilariae, third- and fourth-stage larvae, and in some instances of continuous use, adult heartworms (McCall et al, 2001, 2008b). Because their filaricidal effect on precardiac larvae can be achieved by brief pulsing at very low doses, they have excellent therapeutic/toxic ratios. Macrocyclic lactones, when given according to label instructions, are highly effective and are among the safest medications used in veterinary medicine.

All orally and topically administered macrocyclic lactone chemoprophylactic products are labeled for a 30-day dosing interval. Beyond this interval efficacy against late fourth-stage larvae declines and is unpredictable (Paul et al, 1986). Juvenile worms, which can be found as early as 52 days post infection, are even less susceptible to chemoprophylaxis. As worms age, they require progressively longer-term administration to achieve a high level of protection (McCall, 2005; McCall et al, 2001). The extended post-infection efficacy of macrocyclic lactones is a partial safeguard in the event of inadvertent delay or omission of regularly scheduled doses but does not justify lengthening the recommended one-month interval of administration for the oral and topical formulations. The extent of efficacy against late fourth-stage larvae and juvenile worms has important implications for chemoprophylaxis in dogs that have either missed doses during the transmission season, or are already into the transmission season before chemoprophylaxis is started and may already be infected. Continuous, year-round administration of heartworm preventive is critical in most, if not all, areas of the United States.

Some Collies and other P-glycoprotein–deficient dogs are unusually sensitive to a variety of commonly used veterinary drugs, including some antidepressants, antimicrobial agents, opioids, immunosuppressants, and cardiac drugs *(see sidebar)*. The macrocyclic lactones are also included in this list with toxicities being reported with overdosing or in combination with other P-glycoprotein–inhibiting drugs (Pulliam et al, 1985). These intoxications have occurred most often when concentrated livestock preparations of macrocyclic lactones are either accidentally ingested or overdosed because of human error in dosage calculation. This practice is an "off-label" use of the drugs and is discouraged. The standard chemoprophylactic dosages of all macrocyclic lactones have been shown to be safe in all breeds (Mealey, 2008).

Oral administration: Ivermectin and milbemycin oxime are available for monthly oral administration. Some formulations are flavored and chewable to increase patient acceptance and facilitate administration. Dose units are packaged for dogs within prescribed weight ranges. **To be maximally effective, heartworm prophylaxis should be given year-round**, but if seasonal treatment is chosen, administration should begin at least one month prior to the anticipated start of heartworm transmission and, depending on the product used, may need to be continued for up to 6 months after transmission typically ceases (see section on Lack of Efficacy).

Some Drugs and Other Substances That Inhibit P-Glycoproteins

Antidepressants
- Fluoxetine
- St. John's Wort
- Paroxetine

Antimicrobial Agents
- Erythromycin
- Itraconazole
- Ketoconazole

Opioids
- Methadone
- Pentazocine

Cardiac Drugs
- Verapamil
- Amiodarone
- Quinidine
- Nicardipine

Immunosuppressants
- Cyclosporine
- Tacrolimus

Miscellaneous
- Bromocriptine
- Chlorpromazine
- Tamoxifen
- Grapefruit juice

(Source: http://www.vetmed.wsu. edu/depts-vcpl/drugs.aspx)

Topical administration: Moxidectin and selamectin are available as topically applied liquid formulations. The parameters for treatment with topical products are the same as for monthly oral chemoprophylaxis.

Parenteral administration: A single dose of the slow-release (SR) formulation of subcutaneously injected moxidectin-impregnated lipid microspheres provides continuous protection for 6 months, with the potential to enhance compliance. Treatment every 6 months is recommended for maximal protection.

Reports of Lack of Efficacy

Lack of efficacy (LOE) of a heartworm preventive product is considered by the Center for Veterinary Medicine (CVM) of the US Food and Drug Administration (FDA) as a dog testing heartworm positive regardless of appropriateness of dosage or administration consistency. There are many possible reasons for reports of LOE, including failure to administer sufficient preventive, failure to administer the preventive when it should be given, failure of a dog to retain a dose, and failure of absorption of the active ingredient. There is also biological variation between hosts in drug metabolism and immune response, and in parasite susceptibility to the drug. Thus, the exact cause of a reported LOE can be difficult or impossible to determine.

Fortunately most LOE claims are explained by compliance failure, either between the clinic and the client or the client and the pet, rather than product failure. It is possible for an animal to become infected because of skipped or delayed administration of just one preventive dose, particularly in highly endemic areas. Such areas typically have warm temperatures most of the year, an abundance of standing water, and substantial mosquito populations. These endemic areas also have large populations of infected dogs and wild canids providing a reservoir of infection. Another consideration for LOE reports may include improved sensitivity of heartworm antigen tests over time possibly resulting in detection of more animals with low female worm burdens.

When considering the possibility of resistance, it is generally accepted that genetic polymorphism has always existed in populations of heartworms and resistance-contributing alleles on a gene, or multiple genes exist that could lead to a decrease or loss of susceptibility to macrocyclic lactones (Bourguinat et al, 2011b). What is not known is the frequency of these resistance-contributing alleles, the number of genes involved, and whether these alleles are dominant or recessive in expressing the resistant phenotype. The phenomenon of developing resistance in a population is much more complex than merely the presence of resistant alleles in individuals. Other factors to consider are the distinct biology of the parasite, the extent of refugia (untreated population of hosts), the relative fitness of the wild-type (susceptible) and resistant genotypes in the absence and presence of macrocyclic lactones, the number of animals treated, and the drug dosage used. Product use under specific off-label conditions has been shown to genetically select for worms with relative resistance (Blagburn et al, 2013). These surviving worms over generations can become a resistant subpopulation.

In vitro assays have identified microfilariae that are less susceptible to high doses of all the macrocyclic lactones (Blagburn et al, 2010, 2011). These microfilariae exhibit an allele on the P-glycoprotein gene that differs from the general population. Subsequent in vitro larval migration inhibition assays (LMIA) utilizing L3 derived from these same isolates of microfilariae have demonstrated no significant difference in susceptibility of these propagated isolates as compared with known susceptible isolates (Evans, 2011). This suggests that either the LMIA is measuring a phenotype that is not associated with resistance, the isolates tested from these prophylaxis failures are not resistant, or other unknown factors are involved.

Several published studies examined the susceptibility of the MP3 isolate (also referred to as the MP3 strain[1]) originally collected in northeast Georgia to various heartworm preventives. One study compared the efficacy of a single oral dose of ivermectin and milbemycin at standard prophylactic doses following an experimental infection with 50 MP3 L3 in groups of 14 purpose-bred laboratory dogs (Snyder et al, 2011b). A single adult worm was

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[1]The term *strain* is being used to describe populations of worms being maintained in laboratories. More appropriately, these populations should be described as *propagated isolates*. These populations consist of numerous male and female worms, each with unique genetic makeup, undergoing sexual reproduction leading to production of offspring with their own unique genetic design. Strains more appropriately describe the result of asexually derived populations, such as bacteria.

recovered in both the ivermectin- and milbemycin-treated groups out of a possible 700 L3. A second study compared the efficacy of a single oral dose of ivermectin or milbemycin or a topical dose of moxidectin or selamectin, again at standard prophylactic doses, following an experimental infection with 100 L3 in groups of 8 dogs (Blagburn et al, 2011). In this second study, 7 of 8 dogs had 23 to 24 worms out of a possible 800 L3 recovered from the ivermectin, milbemycin, and selamectin groups. No worms were recovered from the moxidectin group. A third study utilizing the MP3 isolate tested three monthly doses of milbemycin after experimental infection with 40 L3 in 10 dogs (Snyder et al, 2011a). No worms were recovered in any of these 10 dogs.

Looking at these three studies collectively, it is evident the MP3 isolate had decreased susceptibility to single monthly doses of ivermectin, milbemycin, and selamectin but was susceptible to three consecutive monthly doses of milbemycin and a single dose of topical moxidectin. Of greater interest was the 20-fold increase in number of worms recovered when the number of L3 injected was doubled. This would lead one to hypothesize that challenge rates could be involved in LOE and the problems seen in the Mississippi River Valley (MRV) are multifactorial. Genetically, the MP3 isolate does not exhibit the same allele on the P-glycoprotein gene that was noted in the field isolates from the MRV, whose microfilariae had decreased susceptibility to macrocyclic lactones, suggesting multiple genes may be involved (Bourguinat et al, 2011a).

Several in vivo studies were reported in which microfilariae collected from heartworm-infected dogs—many of which were on preventive, had received microfilaricidal doses of macrocyclic lactones and therefore were preselected for macrocyclic lactone resistance—were fed to mosquitoes (Bowman et al, 2013; Kaminsky et al, 2013; Pulaski et al, 2013, 2014). The L3 were then collected and subsequently injected into laboratory dogs, which were then placed on various preventives. These studies identified the presence of resistant subpopulations of heartworms in these dogs. Every compound currently marketed in every form of administration (oral, topical, and parenteral) was less than perfect in at least one study. It seems that although this resistance affects all macrocyclic lactones, differences in active ingredients, doses, and product formulation among the available

preventives can result in varying rates of failures (Blagburn et al, 2013).

Another possible factor in LOE is the host–parasite relationship. The exact mode of action of the macrocyclic lactones at preventive doses is not fully known. A study involving *Brugia malayi*, a filarial nematode causing lymphatic filariasis in humans, indicates that ivermectin disrupts the ability of the parasite to secrete an immunomodulating protein from the secretory vesicle, exposing microfilariae to the host immune response (Moreno et al, 2010). This finding suggests that macrocyclic lactones might work in conjunction with the host immune system to eliminate the microfilariae of *Brugia*. A separate study, utilizing *Dirofilaria immitis* microfilariae, demonstrated attachment of leukocytes to microfilariae in whole blood in the presence of ivermectin (Vatta et al, 2014). No such attachment of white blood cells to microfilariae in untreated whole blood was noted. Additionally, the investigators found no attachment of cells to microfilariae, in the presence of ivermectin, when no serum was present. In separate studies, these same cellular attachment tendencies have been observed with *D immitis* larvae (Abraham and Grieve, 1990; Abraham et al, 1988). These data collectively lead us to believe ivermectin, and likely the other ML products, act by affecting the *D immitis* microfilariae and larvae's ability to inhibit immune recognition, exposing them to immunologic clearance.

Research is ongoing to determine the reason for LOE located predominantly in the MRV. Every new study adds to our knowledge base and increases our understanding but also produces new questions. The complex biology of the parasite, the effect of changing environmental conditions that affect vector populations, the dynamics of host (wild and domestic) populations, and even the dynamics of human interactions with pets are also relevant. In the face of the many variable factors, it is critical that all members of the veterinary practice ensure that clients understand the implications of heartworm infection and the risk of heartworm infection in their area, and that they are providing their pets with appropriate year-round heartworm prevention. The macrocyclic lactones continue to be the best and only option for preventing heartworm infections and efforts need to be intensified in increasing the number of dogs receiving chemoprophylaxis. Reminder systems need to be implemented to assist pet owners in purchasing and administering products in a timely manner.

It is now generally accepted that isolated instances of resistant heartworms have been identified. The extent, the degree of spread, and the reasons for resistance are not understood and are controversial. All agree that owner compliance is the biggest factor in the "failure" of preventives. There is general agreement that resistance to heavy experimental infections is worrisome, and that the products now available are highly effective and should continue to be used as the manufacturers suggest.

PRIMARY DIAGNOSTIC SCREENING

Annual testing is an integral part of ensuring that prophylaxis is achieved and maintained. Should an infection be diagnosed, more timely treatment can be provided to minimize pathology and the potential selection of resistant subpopulations.

Test Timing for Optimal Results

Currently available heartworm antigen tests detect protein secreted mainly by adult female *Dirofilaria immitis* (Courtney and Cornell, 1990), and the most useful microfilaria tests concentrate microfilariae (modified Knott or filtration test) and allow for greater sensitivity (Georgi and Georgi, 1992; Knott, 1939). The earliest that heartworm antigen and microfilariae can be detected is about 5 and 6 months post infection, respectively. Antigenemia usually precedes but sometimes lags the appearance of microfilariae by a few weeks. Antigen may never be detected or may only be sporadically detected in dogs with very low female worm burdens (Atkins, 2003; McCall, 1992). In addition, antigenemia may be suppressed until about 9 months post infection in infected dogs receiving macrocyclic lactone chemoprophylaxis (McCall et al, 2001). To determine when testing might become useful, a pre-detection period should be added to the approximate date on which infection may have been possible. A reasonable interval is 7 months. Thus, there is no need or justification for testing a dog for antigen and microfilariae prior to 7 months of age.

Microfilaria and Antigen Testing

Whether screening a population of asymptomatic dogs or seeking verification of a suspected heartworm infection, antigen testing is the most sensitive diagnostic method. It is now recommended, however, that microfilaria testing be done in tandem with antigen testing. This is especially important if there is a high degree of suspicion or if the heartworm prevention history is unknown (eg, dogs adopted from shelters). It

has come to light that in some dogs infected with heartworms, antigen–antibody complexes may lead to false-negative antigen test results. These dogs will be antigen negative and microfilariae positive; a study conducted on shelter dogs in the southeastern United States reported this occurred at a rate of 7.1% (Velasquez et al, 2014). It is important that these dogs are identified and treated to decrease the potential for selection of resistant subpopulations of heartworms. There will be instances where an infected dog is both antigen and microfilariae negative.

Antigen Tests

Enzyme-linked immunosorbent assay (ELISA) and immunochromatographic test systems are available for detecting circulating heartworm antigen. Each testing format has proven to be clinically useful. The current generation of heartworm antigen tests identifies most "occult" (adult worms present but no circulating microfilariae) infections consisting of at least one mature female worm and are nearly 100% specific (Atkins, 2003; Courtney and Zeng, 2001; Lee et al, 2011; McCall et al, 2001). Differences in sensitivity exist especially in cases with low worm burdens and/or low antigenemia. Currently there are no verified tests capable of detecting infections consisting of only adult male worms.

To obtain reliable and reproducible results, antigen tests must be performed in strict compliance with the manufacturer's instructions. Accuracy of all heartworm tests under field conditions is influenced by adherence to the instructions and storage and handling of the test kit and sample. This has been simplified for several tests that use devices that minimize the number of steps and partially automate the procedure. False-negative and false-positive results can occur. If a test result is unexpected, the test should be repeated. If the result is still ambiguous, independent confirmation by a reference laboratory is recommended to confirm the result. Concentration tests for microfilariae, thoracic radiography to detect signs of heartworm disease, or ultrasonographic visualization of worms may also validate weakly positive antigen test results. In cases of minimal exposure, it is recommended to confirm all positive antigen tests in asymptomatic dogs prior to any adulticide therapy.

The color intensity of a positive antigen test result cannot reliably be used to determine the level of worm burden. The amount of antigen in circulation bears a direct, but imprecise, relationship to the

number of mature female heartworms (Courtney, 1987). A graded test reaction can be recognized by ELISA test systems, but quantitative results are not displayed by immunochromatographic tests. The utility of the ELISA tests for assessing the degree of parasitism is limited by confounding complications such as the transient increase in antigenemia associated with recent worm death or low antigen levels from infections with young adult female worms and/or only a few adult females (Grieve and Knight, 1985; Wang, 1998). Therefore, quantitative analysis of antigen results is highly speculative and requires correlation with other relevant information. For example, radiographic evidence of advanced pulmonary arterial disease typical of chronic heartworm disease coupled with a low or absent antigenemia is consistent with the aftermath of a previous infection that has been cleared, either naturally or by treatment.

False-negative test results occur most commonly when infections are light, female worms are still immature, only male worms are present, and/or the test kit instructions have not been followed. There are also documented cases of antigen–antibody complexes interfering with antigen testing, resulting in false-negative tests. Laboratory studies have shown that heating serum will break down these complexes, release antigen, and result in more accurate test results (Velasquez et al, 2014). The routine heating of blood samples IS NOT RECOMMENDED at this time as this is contrary to the label instructions for these tests. It also could interfere with the results of combination tests that include an antibody test for detection of other infectious agents. Due to this possible interference, and the other considerations mentioned, heartworm test results should only be recorded as positive or no antigen detected (NAD) and should not be written as "negative." Antigen test results should be interpreted carefully, taking other relevant clinical information into consideration. In general, however, it is better to trust rather than reject positive antigen test results.

Microfilaria Tests

In areas where the prevalence of heartworm infection is high, many (~20%) heartworm-infected dogs may not be microfilaremic, and this figure is even higher for dogs on a macrocyclic lactone prevention program (McCall, 2005). Considering this, most microfilaremic dogs can be detected by microscopically examining a drop of fresh

blood under a cover slip for microfilariae or cell movement caused by the motile microfilariae (Rawlings, 1986). A stationary rather than a migratory pattern of movement is indicative of a *Dirofilaria* species, nearly always *D immitis* in the United States. Movement beneath the buffy coat in a microhematocrit tube also may be visible. These are insensitive testing methods when low numbers (50–100/mL) of microfilariae are present; however, such patients are at a lower risk for severe reaction after the administration of a microfilaricide and are less likely to pose a threat as a reservoir of infection. For more accurate results a concentration technique (modified Knott test or filtration test) should be used to determine the absence or presence of microfilariae (Georgi and Georgi, 1992; Knott, 1939). The modified Knott test remains the preferred method for observing morphology and measuring body dimensions to differentiate *D immitis* from non-pathogenic filarial species, such as *Acanthocheilonema* (formerly *Dipetalonema*) *reconditum*.

The modified Knott test is performed by mixing 1.0 mL of EDTA blood with 9.0 mL of 2% formalin in a centrifuge tube. The tube is inverted several times to mix the blood with the formalin solution, lysing the red blood cells. The tube is then placed in a centrifuge, spun at 1100 to 1500 rpm for 5 to 8 minutes, and the liquid is poured off leaving the sediment. A drop of methylene blue is added to the sediment and then the stained sediment is placed on a glass slide and a cover slip applied. The slide is examined under low power (100X) for the presence of microfilariae. To observe the characteristics of the microfilariae, the slide can be examined under high-dry (400X). The microfilariae of *Dirofilaria immitis* are 295 to 325 microns (μm) long and have tapered heads. The microfilariae of *Acanthocheilonema reconditum* are 250 to 288 μm long with blunt heads and curved tails (Figure 3) (Rawlings, 1986).
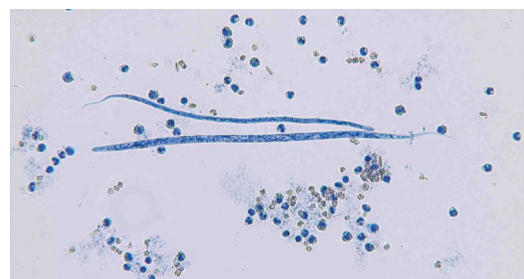
Figure 3. *Acanthocheilonema reconditum* (top) and *Dirofilaria immitis* (below). Photograph courtesy of Byron Blagburn, PhD.

All dogs should be tested for microfilariae. Microfilaremia validates serologic results, is diagnostic should a dog have antigen–antibody complexes (no antigen detected on antigen tests), identifies the patient as a reservoir of infection, and alerts the veterinarian to a high microfilariae burden, which may precipitate a severe reaction following administration of a microfilaricide.

Testing Considerations Following Noncompliance and When Changing Products

In instances of noncompliance or changing the brand or type of heartworm preventive, it is important to determine the heartworm status of the dog. The dog should be antigen and microfilaria tested prior to starting or changing products. A positive test indicates preexisting infection. The dog should *always* be retested 6 months later (Figure 4). A positive test at this time would most likely be due to an infection acquired before starting or resuming preventive therapy; however, in rare instances, an existing infection might be missed (ie, false-negative test due mainly to young or low worm burden infection). Antigen and microfilaria testing should be performed on the one-year anniversary date of the initial test and annually thereafter.

OTHER DIAGNOSTIC AIDS

Additional testing methods are useful for confirming the diagnosis and staging the severity of heartworm disease.

Radiography

Assessment of cardiopulmonary status may be useful for evaluating a patient's prognosis. Radiography provides the most objective method of assessing the severity of heartworm cardiopulmonary disease secondary to heartworm infection. Typical (nearly pathognomonic) signs of heartworm vascular disease are enlarged, tortuous, and often truncated peripheral intralobar and interlobar branches of the pulmonary arteries, particularly in the diaphragmatic (caudal) lobes

(Figure 5). These findings are accompanied by variable degrees of pulmonary parenchymal disease. The earliest and most subtle pulmonary arterial changes are most commonly found in the dorsal caudal wedge of the diaphragmatic lung lobes. As the severity of infection and chronicity of disease progress, the pulmonary arterial signs are seen in successively larger branches (Figure 6). In the worst cases, the right heart eventually enlarges (Bowman and Atkins, 2009; Calvert and Rawlings, 1988; Rawlings, 1986).

Echocardiography

The body wall of adult heartworms is highly echogenic and produces distinctive, short parallel-sided images with the appearance of "equal signs" where the imaging plane cuts across loops of the parasite. Echocardiography can provide definitive evidence of heartworm infection, as well as allow for assessment of cardiac anatomic and functional consequences of the disease (Figure 7). It is not an efficient method of making this diagnosis, however, particularly in lightly infected dogs, because the worms often are limited to the peripheral branches of the pulmonary arteries beyond the echocardiographic field of view. When heartworms are numerous, they are more likely to be present in the main pulmonary artery, right and proximal left interlobar branches, or within the right side of the heart where they can be imaged easily. In dogs with hemoglobinuria, visualization of heartworms in the orifice of the tricuspid valve provides conclusive confirmation of caval syndrome (Badertscher et al, 1988; Moise, 1988; Venco et al, 2001).

PRE-ADULTICIDE EVALUATION

The extent of diagnostic testing necessary in the pre-adulticide evaluation varies depending on the clinical status of each patient. Selected clinical and laboratory tests should only be performed to complement information obtained from a thorough history, physical examination, and antigen and microfilaria tests. It is important to note that some

	6 months		6 months		1 year	
Noncompliance: Test Day 0	→	Test Day 180	→	Test Day 360	→	Test annually thereafter

Figure 4. The testing protocol following known noncompliance includes three tests in the first year, with annual testing thereafter.
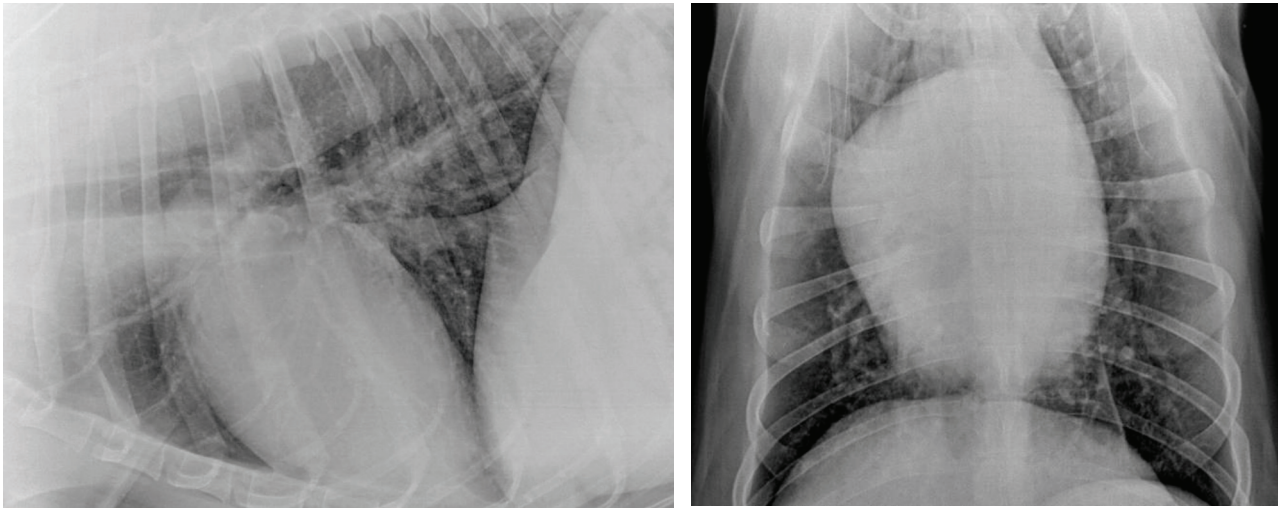
Figure 5. Moderate heartworm disease. Radiographic images courtesy of C. Thomas Nelson, DVM.
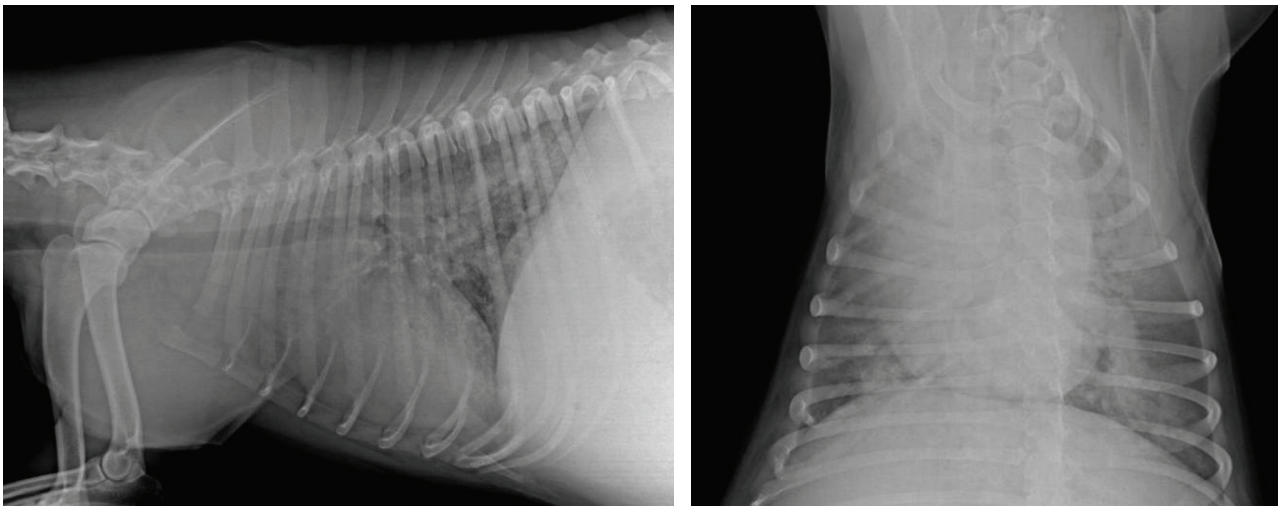
Figure 6. Severe heartworm disease. Radiographic images courtesy of C. Thomas Nelson, DVM.

key factors influencing the probability of post-adulticide thromboembolic complication and outcome of treatment are not easily measured with standard diagnostic procedures, including 1) the activity level of the dog, 2) the extent of concurrent pulmonary vascular disease, and 3) the severity of infection (high versus low worm burdens).

High activity levels of the dog are one of the most significant factors contributing to post-adulticidal complications (Dillon et al, 1995; Fukami et al, 1998). Prior to treatment, the owner's ability and willingness to properly confine treated dogs should be thoroughly investigated. Restricting activity is imperative as exercise, excitement, and overheating are harbingers of complications.

Thoracic radiographs can assist in providing an assessment of the animal's cardiopulmonary status and can be helpful in evaluating the potential for

post-adulticide treatment complications (Calvert and Rawlings, 1988; Rawlings, 1986). Thromboembolic disease is commonly seen in infected dogs exhibiting radiographic signs of severe pulmonary arterial obstruction, especially in those animals presenting with clinical signs (Rawlings et al, 1993b). Regardless of radiographic findings, heartworms must be eliminated, although not necessarily immediately, in all patients that can tolerate the death of worms.

The greater the number of heartworms killed during an adulticide treatment, the more significant the potential for obstructive and inflammatory pathology (Venco et al, 2004). Unfortunately, no test (or combination of tests) is available to accurately determine the number of heartworms present. Whether carrying low or high worm burdens, infected dogs can be clinically asymptomatic and have minimal radiographic changes. So, even with
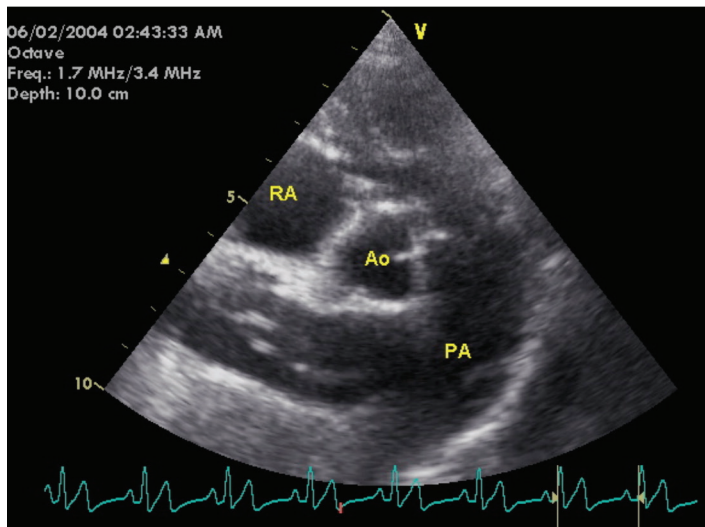
Figure 7. Echocardiogram image courtesy of Matthew Miller, DVM.

extensive diagnostics, predicting post-adulticide complications is difficult. One must always assume post-treatment complications are likely, and every infected pet must be managed as though a substantial heartworm mass is present or a potently violent individual immune reaction to the dead and dying worms could occur.

Historically, due to financial limitations of some pet owners and animal shelters, large numbers of adulticide treatments have been successfully performed without the benefit of extensive diagnostics. While diagnostics can be an important part of defining an individual's heartworm disease status, each plan must be developed considering both the animal and individual pet owner. No set protocol has been established for pre-treatment workup and reasonable judgment should always be used to weigh the necessity, benefit, and extent of each diagnostic procedure performed.

Adult heartworms are a grave risk to our canine patients. The longer they remain in an animal, the greater the damage to the cardiopulmonary system and the greater the risk of illness and death. It is probable that treating in the absence of diagnostics, while not ideal, is better than refusing to perform a needed treatment.

PRINCIPLES OF TREATMENT

Treating heartworm infections in asymptomatic patients or those exhibiting signs of mild disease usually is not problematic if exercise is curtailed. Infections associated with moderate or severe heartworm disease (Table 1) or in patients with

concurrent disease often are challenging.

The goals of any heartworm treatment are to improve the clinical condition of the animal and to eliminate all life stages of the heartworms (microfilariae, larval stages, juveniles, and adults) with minimal post-treatment complications. Dogs exhibiting significant clinical signs of heartworm disease should be stabilized before administering an adulticide. This may require administration of glucocorticosteroids, diuretics, vasodilators, positive inotropic agents, and fluid therapy.

A thorough understanding of the host–parasite relationship is necessary to effectively manage all cases. As expected, the number of worms has an effect on the severity of disease; but of equal, if not greater, importance is the activity level of the dog. Controlled studies have shown that dogs infected by surgical transplantation with 50 heartworms and exercise-restricted took longer to develop clinical disease and developed less pulmonary vascular disease than dogs with 14 heartworms and allowed moderate activity (Dillon et al, 1995). This was also evident in a study in naturally infected dogs where there was no correlation between the number of heartworms and pulmonary vascular resistance and is an indication that the host–parasite interaction plays a significant role in the severity of disease (Calvert, 1986). A subsequent study reported similar findings in dogs being treated with melarsomine (Fukami et al, 1998).

Whereas live heartworms can cause endarteritis and muscular hypertrophy of arteriolar walls, primarily of the caudal pulmonary arteries, dying and dead heartworms cause a significant portion of pathology seen in clinical disease. As worms die from either natural causes or as a result of administration of adulticidal therapy, they decompose; and worm fragments lodge in the distal pulmonary arterioles and capillary beds in the caudal lung lobes, blocking blood flow. These worm fragments along with the elicited inflammation and platelet aggregation result in thromboembolisms. During periods of increased activity or exercise, the increased blood flow to these blocked vessels can cause capillary delamination, rupture, and subsequent fibrosis (Case et al, 1995; Dillon et al, 1995; Hoskins et al, 1985; Rawlings et al, 1993a). This leads to increased pulmonary vascular resistance and potentially, right-sided heart failure and illustrates a direct correlation between the activity level of the dog and the severity of disease.

Table 1. Summary of Clinical Signs of Canine Heartworm Disease

Mild	Asymptomatic or cough
Moderate	Cough, exercise intolerance, abnormal lung sounds
Severe	Cough, exercise intolerance, dyspnea, abnormal heart and lung sounds, enlarged liver (hepatomegaly), syncope (temporary loss of consciousness from reduced blood flow to the brain), ascites (fluid accumulation in the abdominal cavity), death
Caval Syndrome	Sudden onset of severe lethargy and weakness accompanied by hemoglobinemia and hemoglobinuria

ADULTICIDE THERAPY

Melarsomine Dihydrochloride

Melarsomine, administered via deep intramuscular injection into the belly of the epaxial lumbar muscles (between L3 and L5), is the only adulticidal drug approved by the FDA. Mild swelling and some soreness at the injection site may be present for a few days, but this can be minimized by ensuring that the injection is deposited into the belly of the epaxial musculature with a needle newly changed after the drug is drawn into the syringe and of appropriate length and gauge for the size of dog and body condition. Strictly adhering to the manufacturer's instructions for administration is imperative. Exercise restriction during the recovery period is ESSENTIAL for minimizing cardiopulmonary complications (see Pulmonary Thromboembolism).

Melarsomine had not been shown to have activity against worms less than 4 months old (Dzimianski et al, 1990; Dzimianski et al, 1989); recent unpublished data, however, suggest that melarsomine may have more efficacy against juvenile worms than previously believed (McCall et al, 2010). The two-injection protocol with melarsomine (ie, two injections of 2.5 mg/kg body weight 24 hours apart) listed on the product insert for treating class 1 and 2 heartworm disease kills only about 90% of the adult worms. The three-dose alternate protocol (one injection of 2.5 mg/kg body weight followed at least one month later by two injections of the same dose 24 hours apart) listed for treating class 3 heartworm disease kills 98% of the worms (Keister et al, 1992; Vezzoni et al, 1992). These overall efficacy values reflect the percentage of worms killed in groups of dogs and not the percentage of dogs cleared of worms, which are considerably lower than these overall efficacy values. The three-dose protocol has the added advantage of decreased complication rates and increased safety as a number of the adult worms are killed with the first melarsomine injection and most, if not all, of the remaining worms are killed with the second and third injections.

Staging of the disease and use of the two-dose protocol has failed to adequately ensure treatment success. Therefore, regardless of the severity of the disease (with the exception of caval syndrome), the three-dose protocol is recommended by the American Heartworm Society due to the increased safety and efficacy.

Pulmonary Thromboembolism

Pulmonary thromboembolism is an inevitable consequence of successful adulticide therapy and may be severe if infection is heavy and pulmonary arterial disease is extensive. If signs of embolism (low grade fever, cough, hemoptysis, exacerbation of right heart failure) develop, they are usually evident within 7 to 10 days, but occasionally as late as 4 weeks after completion of adulticide administration (Hirano et al, 1992). Mild embolism in relatively healthy areas of lung may be clinically unapparent. A pivotal factor in reducing the risk of thromboembolic complications is STRICT exercise restriction.

ADJUNCT THERAPY

Steroids

Administration of diminishing anti-inflammatory doses of glucocorticosteroids helps control clinical signs of pulmonary thromboembolism (Atwell and Tarish, 1995). Whereas studies showed a decrease in efficacy of the arsenical thiacetarsamide when glucocorticosteroids were administered concurrently (Rawlings et al, 1984), a study showed no decrease in the efficacy of melarsomine when used in conjunction with prednisone (Dzimianski et al, 2010). In highly endemic areas where animals are more likely to have significant worm burdens, glucocorticosteroids such as prednisone may be
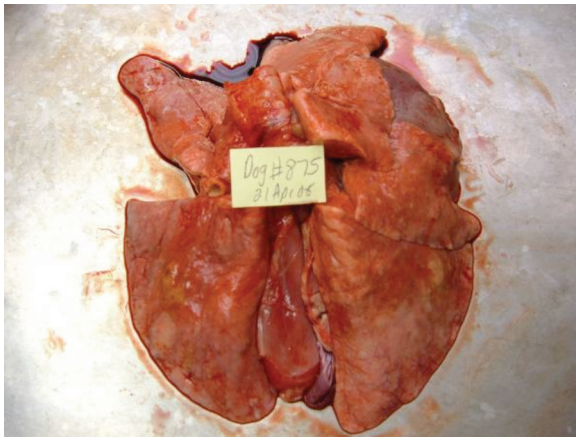
Melarsomine only
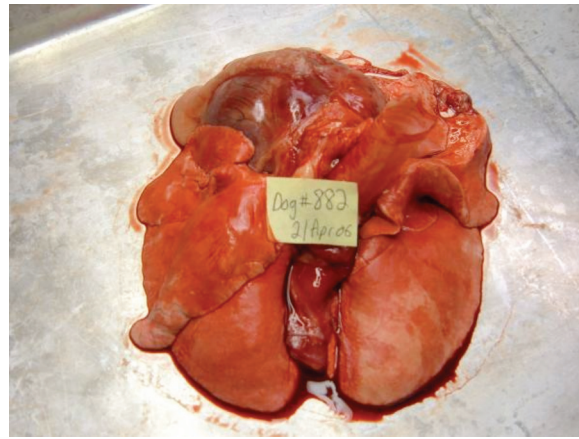
Ivermectin / Doxycycline / Melarsomine
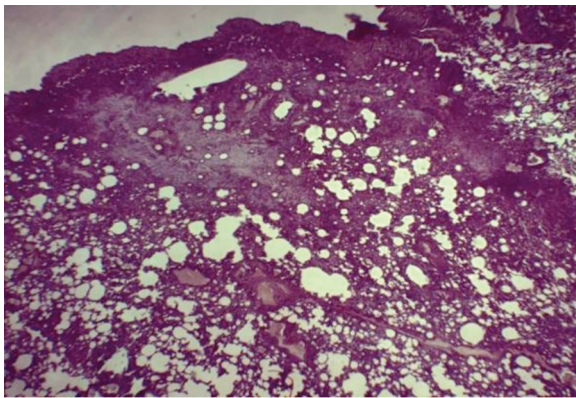
Melarsomine only

Ivermectin / Doxycycline / Melarsomine

Figure 8. Pulmonary pathology associated with the death of heartworms in experimentally infected heartworm-positive dogs pretreated with ivermectin and doxycycline prior to receiving melarsomine injections. Photographs courtesy of John McCall, PhD and Laura Kramer, DVM, PhD.

used. Prednisone is routinely dosed at 0.5 mg/kg twice a day (BID) for the first week and 0.5 mg/kg once daily (SID) for the second week, followed by 0.5 mg/kg every other day (EOD) for 1 to 2 weeks.

NSAIDs/Aspirin

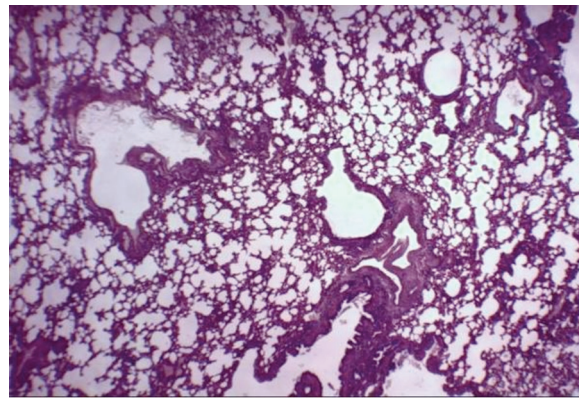The empirical use of aspirin for its antithrombotic effect or to reduce pulmonary arteritis is not recommended for heartworm-infected dogs (Boudreaux et al, 1991). Convincing evidence of clinical benefit is lacking and there is some research suggesting that aspirin may be contraindicated.

Doxycycline

Many filarial nematodes, including *Dirofilaria immitis*, harbor obligate, intracellular, gram-negative, endo-symbiotic bacteria belonging to the genus *Wolbachia* (*Rickettsiales*) (Kozek, 2005; Taylor et al, 2005). Doxycycline reduces *Wolbachia* numbers in all stages of heartworms. Doxycycline administration during the first or second month following experimental heartworm infection was

lethal to third- and fourth-stage heartworm larvae (McCall et al, 2011). In addition, in dogs with adult infections, doxycycline gradually suppressed microfilaremia (Bazzocchi et al, 2008; McCall et al, 2008a). Microfilariae ingested by mosquitoes on dogs treated with doxycycline developed into third-stage larvae that appeared to be normal in appearance and motility, but these larvae were not able to develop into adult worms, thus reducing the risk of selecting for resistant subpopulations (McCall et al, 2008a, 2014b).

Wolbachia have also been implicated as a component in the pathogenesis of filarial diseases, possibly through their metabolites (Bouchery et al, 2013; Kramer et al, 2005). Recent studies have shown that a major surface protein of *Wolbachia* (WSP) induces a specific IgG response in hosts infected by *D immitis* (Kramer et al, 2005). It is hypothesized that *Wolbachia* may contribute to pulmonary and renal inflammation through its surface protein WSP. Studies have shown that experimentally infected heartworm-positive dogs pretreated with ivermectin
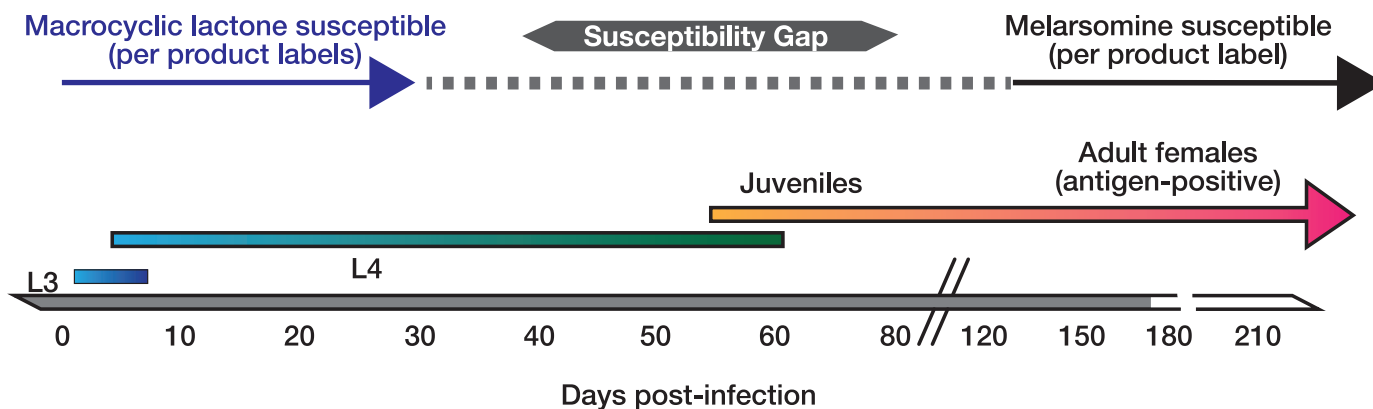
Figure 9. Timeline of *D immitis* development, showing periods of susceptibility to macrocyclic lactones and melarsomine. The dotted line represents the "treatment gap," when *D immitis* is not considered to be susceptible to either treatment. From Merial Limited, Duluth, GA. ©2008. All rights reserved.

and doxycycline prior to receiving melarsomine injections had less pulmonary pathology associated with the death of the heartworms (Figure 8) (Kramer et al, 2011; McCall et al, 2008a).

When incorporated into a heartworm treatment protocol, doxycycline should be given before administration of melarsomine so the *Wolbachia* organisms and their metabolites are reduced or absent when the worms die and fragment. Doxycycline is administered at 10 mg/kg BID for 4 weeks. Doxycycline has been shown to eliminate over 95% of the *Wolbachia* organisms in the filarial nematode *Wuchereria bancrofti*, resulting in amicrofilaremia for 12 months (Hoerauf et al, 2003). These data suggest the absence of *Wolbachia*, or at least very low numbers, as the organism is necessary for embryogenesis. In *D immitis* (adults and microfilariae) data suggest *Wolbachia* numbers remain low for at least 12 months following doxycycline administration (Rossi et al, 2010).

Minocycline has been shown to be highly effective in eliminating *Wolbachia* organisms from the filarial nematode *Onchocerca gutturosa* (Townson et al, 2006). No published studies have been conducted in *D immitis* but available pharmacological data and anecdotal reports suggest this is a viable alternative if doxycycline is not available. The dosing regimen is the same as doxycycline.

Macrocyclic Lactones

It is highly probable that a heartworm-positive dog harbors heartworms that can range from less than 1 month to as much as 7 years of age. The incomplete efficacy of melarsomine against young adult worms could present a problem in achieving

the goal of eliminating all of the worms. The susceptibility gap between the macrocyclic lactones and melarsomine is demonstrated in Figure 9.

The susceptibility gap can be minimized by administering a macrocyclic lactone preventive for 2 months prior to administering melarsomine. This will reduce new infections, eliminate existing susceptible larvae, and allow older worms (2 and 4 months of age) to mature to a point where they would be more susceptible to melarsomine. Reduction of the susceptibility gap can also be potentiated with concurrent use of doxycycline for 30 days, as this will essentially eliminate all developing larvae during the first 60 days of infection.

Macrocyclic lactones administered as microfilaricides may cause a rapid decrease in the numbers of microfilariae and should be used with caution in dogs with high microfilarial counts. Pretreatment with antihistamines and glucocorticosteroids will minimize potential reactions. Topical moxidectin is now FDA-approved for use in heartworm-positive dogs to eliminate microfilariae. No adverse reactions due to high microfilarial counts were observed in the laboratory or field studies conducted for approval of this label claim (McCall et al, 2014).

Macrocyclic Lactones/Doxycycline

Select macrocyclic lactones coupled with doxycycline individually suppress embryogenesis and weaken adult heartworms. As mentioned previously doxycycline reduces *Wolbachia* levels in all stages of heartworms. Studies have shown that administration of doxycycline in combination with ivermectin provided more rapid adulticidal activity than ivermectin alone as well as more efficiently

reducing *Wolbachia* numbers more effectively than doxycycline alone (Bazzocchi et al, 2008). Anecdotal reports on other macrocyclic lactones with adulticidal properties suggest similar results but no confirmatory studies have been published.

In cases where arsenical therapy is not possible or is contraindicated, the use of a monthly heartworm preventive along with doxycycline at 10 mg/kg BID for a 4-week period might be considered. An antigen test should be performed every 6 months and the dog not considered cleared until two consecutive NAD (no antigen detected) heartworm antigen tests, 6 months apart, have been obtained. If the dog is still antigen positive after one year, repeat the doxycycline therapy. Exercise should be rigidly restricted for the duration of the treatment process.

AHS-RECOMMENDED TREATMENT PROTOCOL

The AHS recommends a multimodal approach to treating heartworms based on the information presented above and depicted in the following example management protocol (Table 2) (Nelson, 2012).

A retrospective study of clinical cases comparing the protocol listed in Table 2 with a similar protocol without doxycycline showed a decrease in respiratory complications and mortality rates when doxycycline was included (Nelson and Sellers, 2013).

SURGICAL EXTRACTION OF ADULT HEARTWORMS

Caval Syndrome (Dirofilarial Hemoglobinuria)

Caval syndrome develops acutely in some heavily infected dogs when adult heartworms partially obstruct blood flow through the tricuspid valve and also interfere with valve closure. Severe passive congestion of the liver, a coarse systolic murmur of tricuspid regurgitation, and jugular pulsations are characteristic features of the syndrome. The diagnosis is based on a sudden onset of severe lethargy, dyspnea, pale mucous membranes, and weakness accompanied by hemoglobinemia and hemoglobinuria (Atwell and Buoro, 1988; Kitagawa et al, 1986; Venco, 1993). Caval syndrome can be confirmed conclusively by echocardiographic visualization of heartworms within the tricuspid orifice and posterior vena cava (Figure 10) (Atkins et al, 1988). The clinical course usually ends fatally within 2 days if surgical extraction of the worms is not pursued promptly.

Surgical removal of worms from the right atrium and orifice of the tricuspid valve can be accomplished
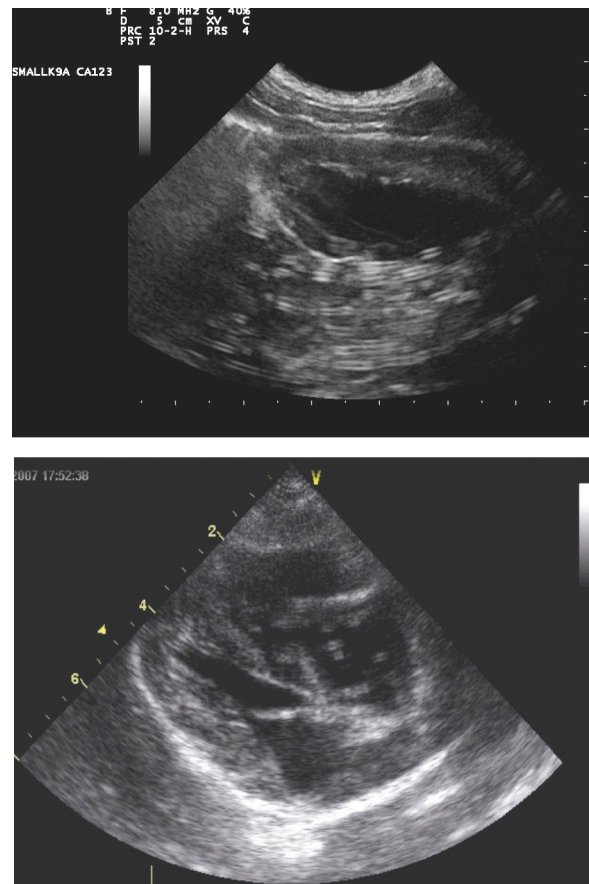
Figure 10. Echocardiogram images courtesy of Stephen Jones, DVM (above) and Matthew Miller, DVM (below).

using light sedation (may not be necessary), local anesthesia, and either a rigid or flexible alligator forceps or an intravascular retrieval snare introduced preferentially via the right external jugular vein (Yoon et al, 2013). With fluoroscopic guidance if available, the instrument should continue to be passed until worms can no longer be retrieved (Figure 11) (Ishihara et al, 1988; Jackson et al, 1977). Immediately following a successful operation, the murmur should soften or disappear and within 12 to 24 hours hemoglobinuria should disappear. Fluid therapy may be necessary in critically ill, hypovolemic dogs to restore hemodynamic and renal function. Within a few weeks following recovery from surgery, adulticide chemotherapy is recommended to eliminate any remaining worms, particularly if many are still visible echocardiographically.
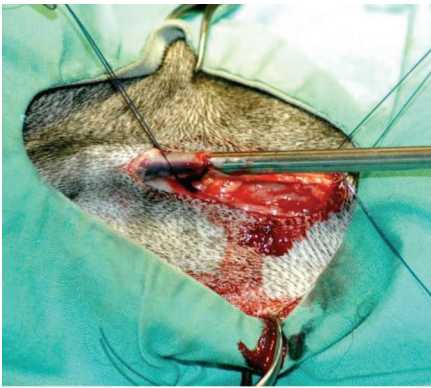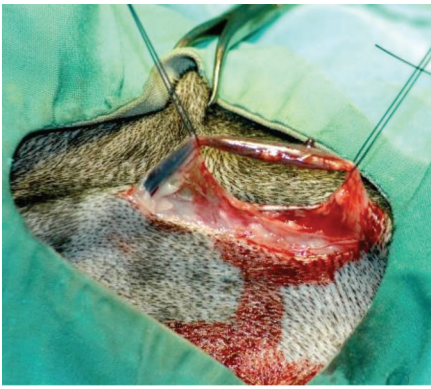
Pulmonary Arterial Infections

The main pulmonary artery and lobar branches can be accessed with flexible alligator forceps aided by fluoroscopic guidance. Intraoperative mortality with this technique is very low. Overall survival and

Table 2. AHS-Recommended Management Protocol

Day	Treatment
Day 0	Dog diagnosed and verified as heartworm positive: • Positive antigen (Ag) test verified with microfilaria (MF) test • If no microfilariae are detected, confirm with 2nd Ag test from a different manufacturer Begin exercise restriction. • The more pronounced the signs, the stricter the exercise restriction If the dog is symptomatic: • Stabilize with appropriate therapy and nursing care • Prednisone prescribed at 0.5 mg/kg BID 1st week, 0.5 mg/kg SID 2nd week, 0.5 mg/kg EOD 3rd and 4th weeks
Day 1	Administer heartworm preventive. • If microfilariae are detected, pretreat with antihistamine and glucocorticosteroid, if not already on prednisone, to reduce risk of anaphylaxis • Observe for at least 8 hours for signs of reaction
Days 1–28	Administer doxycycline 10 mg/kg BID for 4 weeks. • Reduces pathology associated with dead heartworms • Disrupts heartworm transmission
Day 30	Administer heartworm preventive.
Day 60	Administer heartworm preventive. First melarsomine injection 2.5 mg/kg intramuscularly (IM) Prescribe prednisone 0.5 mg/kg BID 1st week, 0.5 mg/kg SID 2nd week, 0.5 mg/kg EOD 3rd and 4th weeks. Decrease activity level even further. • Cage restriction/on leash when using yard
Day 90	Administer heartworm preventive. Second melarsomine injection 2.5 mg/kg IM
Day 91	Third melarsomine injection 2.5 mg/kg IM Prescribe prednisone 0.5 mg/kg BID 1st week, 0.5 mg/kg SID 2nd week, 0.5 mg/kg EOD 3rd and 4th weeks. Continue exercise restriction for 6 to 8 weeks following last melarsomine injections.
Day 120	Test for presence of microfilariae. • If positive treat with a microfilaricide and retest in 4 weeks Establish year-round heartworm prevention.
Day 271	Antigen test 6 months after completion; screen for microfilariae.

Figure 11. Surgical removal of worms. Photographs courtesy of C. Thomas Nelson, DVM.

rate of recovery of dogs at high risk of pulmonary thromboembolism is improved significantly by physically removing as many worms as possible before beginning adulticide therapy (Morini et al, 1998). When the facilities are available, worm extraction is the procedure of choice for the most heavily infected and high-risk dogs. Before electing this method of treatment, however, echocardiographic visualization of the right heart and pulmonary arteries should be performed to determine that a sufficient number of worms are in accessible locations.

ALTERNATIVE THERAPIES

Long-term Macrocyclic Lactone Administration

Slow-kill methods using continuous monthly administration of prophylactic doses of any macrocyclic lactone are NOT RECOMMENDED. While effective in reducing the life span of juvenile and adult heartworms, it appears that the older worms are less susceptible, taking longer to die. The adulticidal effect of macrocyclic lactones has been shown to take more than 2 years of continuous administration before adult heartworms are 95% eliminated, and the timing for rigid exercise restriction is unknown with this approach (McCall et al, 2001). Throughout this period, the infection would persist and pathology would continue to progress (Rawlings et al, 2001). Another important concern in using macrocyclic lactones in monotherapy of heartworm-positive dogs as stand-alone therapy is the potential for selection of resistant subpopulations of heartworms (Bowman, 2012; Geary et al, 2011).

Herbal Therapies

No "natural" or herbal therapies have been shown to be safe and effective prevention or treatment for heartworm disease.

CONFIRMATION OF ADULTICIDE EFFICACY

Clinical improvement is possible without completely eliminating the adult heartworms. Worms that do survive adulticide treatment are invariably the antigen-producing females. Most microfilaremic dogs with post-adulticide, female unisex infections become occult within 6 to 9 months, with or without microfilaricide treatment, and particularly if they were treated with doxycycline and are on a macrocyclic lactone preventive during and after adulticidal therapy (Grandi et al, 2010; McTier et al, 1994). Consequently, clinical improvement and successful clearance of microfilariae from the blood do not verify a complete adulticide effect. Recurrence of microfilaremia 6 months later may be due to incomplete clearance of adult worms, maturation of immature worms if a preventive was not given during adulticide therapy, or a new infection due to a lapse in chemoprophylaxis.

Heartworm antigen testing is the most reliable method of confirming the efficacy of adulticidal therapy. If all of the adult female worms have been killed, heartworm antigen should become undetectable by 6 months post treatment (Maxwell et al, 2014; McTier et al, 1994). However, this single test result does not verify that the dog is negative for heartworms, as larval and/or juvenile heartworms may be present

in the dog and an insufficient amount of antigen is being produced by these young worms to elicit a positive test result. This is especially critical if a macrocyclic lactone was not administered prior to or initiated concurrently with adulticidal therapy. If a heartworm-positive dog is immediately treated with adulticide and a macrocyclic lactone is not given until 3 to 4 weeks after the last dose of adulticide, the dog should have a negative antigen test 7 months after the initial dose of macrocyclic lactone before being considered cleared of adult worms. Since adult worms may continue to die for more than a month following adulticide administration, dogs that are still antigenemic at any time less than 6 months post treatment should be allowed more time to clear antigen before retreatment is considered.

ELIMINATION OF MICROFILARIAE

Macrocyclic lactones administered as microfilaricides may cause a rapid decrease in the numbers of microfilariae and should be used with caution in dogs with high microfilarial counts. Pretreatment with antihistamines and glucocorticosteroids is advisable in the face of high microfilaria burdens to minimize potential reactions (Bowman and Atkins, 2009). Topical moxidectin is approved by the FDA to eliminate microfilariae (McCall et al, 2014). No adverse reactions due to high microfilaria counts were observed in the laboratory or field studies conducted for approval of this label claim.

Historically, microfilaricidal treatment was usually done about 3 weeks to a month after adulticidal therapy, with the understanding that several weekly treatments were often required to completely eliminate circulating microfilariae (McCall et al,

2008b). Current protocols utilizing doxycycline in combination with regular preventive doses of macrocyclic lactones have essentially eliminated the need for post-adulticidal elimination of microfilariae (Bazzocchi et al, 2008; McCall et al, 2008a). Administration of a macrocyclic lactone should always begin as soon as the dog is diagnosed with a heartworm infection. Including doxycycline in the treatment protocol as previously described hastens the elimination of microfilariae.

When elimination of microfilariae is accomplished in the course of heartworm chemoprophylaxis, a microfilaria test should be performed in adulticide-treated dogs at the time the antigen test is conducted 6 months post treatment. Controlling the spread of heartworms entails decreasing the microfilaremic reservoirs of infection in the dog population and the benefits of doing so have been cited (see HEARTWORM CHEMOPROPHYLAXIS).

ELECTIVE SURGERIES ON DOGS WITH HEARTWORMS

Veterinarians are frequently faced with the decision whether to perform an elective procedure, such as a spay or neuter, on a heartworm-positive dog. A study has shown no increase in perioperative complications in heartworm-positive dogs with no to mild clinical signs of heartworm disease (Peterson et al, 2014). Elective surgical procedures should be avoided in dogs exhibiting signs of more advanced disease, and treatment utilizing the protocol in Table 2 should be initiated. Surgery can then be performed 6 months after adulticidal treatment if the dog has recovered sufficiently.

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These guidelines are based on the latest information on heartworm disease. In keeping with the objective of the Society to encourage adoption of standardized procedures for the diagnosis, treatment, and prevention of heartworm disease, they will continue to be updated as new knowledge becomes available.