American Heartworm Society Canine Guidelines for the

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

AMERICAN HEARTWORM SOCIETY
EST. 1974

Revised 2024

Thank You to Our Generous Sponsors

Platinum:

Boehringer Ingelheim

Elanco

MERCK Animal Health

zoetis

Silver:

ceva

IDEXX LABORATORIES

PO Box 1352 | Holly Springs, NC 27540 | USA | E-mail: info@heartwormsociety.org

CONTENTS

Click on the links below to navigate to each section.

Prepared by Dr. C. Thomas Nelson, Dr. John W. McCall, Dr. Andrew Moorhead, Dr. Lindsay Starkey, and Dr. Marisa Ames and approved by the Executive Board of the American Heartworm Society (Officers: Dr. Jennifer Rizzo, President; Dr. Chris Duke, Past President; Dr. Chris Adolph, Vice President; Dr. Angele Bice, Secretary-Treasurer; Dr. Lindsay Starkey, Editor; Dr. C. Thomas Nelson, Research Chair; Dr. Andrew Moorhead, Symposium Program Chair; Board Members: Dr. Marisa Ames, Dr. Blue Brawner, Dr. Elizabeth Clyde, Dr. Mark Cousins, Dr. Uri Donnett, and Dr. Aliya Magee; and Ex Officio Members: Dr. Melissa Bourgeois, Dr. Doug Carithers, Dr. Lisa Young, Dr. John W. McCall, and Paola Domínguez-López, CVT.

Preamble

These recommendations supersede previous editions and are based on the information presented at the 2022 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 (available on the AHS website).

ABBREVIATIONS

AFAST, abdominal focused assessment with sonography for trauma

AHS, American Heartworm Society

AMDUCA, Animal Medicinal Drug Use Clarification Act of 1994

CVM, Center for Veterinary Medicine

EPA, Environmental Protection Agency

FDA, US Food and Drug Administration

L1, first-stage larvae

L2, second-stage larvae

L3, third-stage larvae

L4, fourth-stage larvae

LOE, lack of efficacy

MF, microfilaria

ML, macrocyclic lactone

NAD, no antigen detected

POCUS, point of care ultrasound

PTE, pulmonary thromboembolism

SR, slow release

TFAST, thoracic focused assessment with sonography for trauma

Vet BLUE, veterinary brief lung ultrasound examination

WSP, *Wolbachia* surface protein

HIGHLIGHTS

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

- **Prevention**
  AHS recommends veterinarians prescribe year-round administration of preventive drugs approved by the US Food and Drug Administration (FDA) to prevent heartworm infection and enhance compliance, the latter being particularly important in light of the documented presence of resistant subpopulations. Application of an Environmental Protection Agency (EPA) registered mosquito repellent/ectoparasiticide has been shown to increase the overall efficacy of a heartworm prevention program in laboratory studies involving known resistant heartworm isolates by providing control of the arthropod vector of heartworm. Furthermore, use of an FDA-approved ectoparasiticide product can break the transmission cycle and could aid in reducing mosquito populations. AHS recommends reduction of exposure to mosquitoes through standard environmental control of mosquitoes and their breeding environments, and when possible, reducing outdoor exposure during key mosquito feeding periods.

- **Adulticide Therapy**
  AHS recommends use of doxycycline and a macrocyclic lactone (ML) 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 MLs as a slow-kill adulticide is not recommended.

EPIDEMIOLOGY

KEY POINTS: EPIDEMIOLOGY

- Heartworm infection has been diagnosed in all 50 states and around the globe.
- Environmental and climatic changes, both natural and those created by humans, relocation of microfilaremic dogs, and expansion of the territories of microfilaremic wild canids continue to be important factors contributing to further spread of the parasite.
- A pivotal prerequisite for heartworm transmission is a climate that provides adequate temperature and humidity to support a viable mosquito population, and can also sustain sufficient heat to allow maturation of ingested microfilariae into infective, third-stage larvae (L3) within the intermediate host.
- The length of the heartworm transmission season in the temperate latitudes also depends on factors such as the influence of microclimates, unique biological habits and adaptations of the mosquito vector, variations in time of larval development, mosquito life expectancy, and temperature fluctuations.
- Heartworm transmission does decrease in colder months, but the presence of microenvironments in urban areas suggests that the risk of heartworm transmission never reaches zero.

Heartworm infection in dogs has been diagnosed around the globe. 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, US Virgin Islands, and Guam (Bowman et al., 2009; Kozek et al., 1995; Ludlam et al., 1970). There are regions in Alaska that have mosquito vectors and climate conditions to support the transmission of heartworms for brief periods, and non-native pet dogs have tested positive within the state (Darsie and Ward, 2005; Slocombe et al., 1995; Terrell, 1998; AHS incidence survey, 2022; CAPC prevalence maps, 2023). Thus, the introduction of microfilaremic dogs or wild canids to non-endemic locations could set up a nidus of infection for local transmission of heartworm (see box below for more on the role of transport of infected dogs). Such relocation of microfilaremic dogs and expansion of the territories of microfilaremic wild canids in other areas 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.

Minimizing Heartworm Transmission in Relocated Dogs

Transporting and relocating dogs is an increasingly common practice. Whether the situation is an owned pet accompanying emigrating or traveling caretakers, the relocation of homeless animals for adoption, or the movement of dogs for competition, exhibition, research or sale, this process carries the risk of spreading infectious diseases. This includes the transmission of *Dirofilaria immitis* when infected dogs are microfilaremic.

The American Heartworm Society, in collaboration with the Association of Shelter Veterinarians, has developed a protocol to help minimize the risk of heartworm transmission associated with transportation and relocation of dogs. The document, which includes an algorithm outlining testing and treatment recommendations, is available on the AHS website.

Environmental and climatic changes, both natural 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 home sites. In the Western United States, irrigation and planting of trees has expanded the habitat for *Aedes sierrensis* (western treehole mosquito), a primary vector for transmission of heartworms in those states (Scoles et al., 1993; Scoles & Dickson, 1995).

Aedes albopictus (Asian tiger mosquito), which was introduced into the Port of Houston in 1985, has now spread northward and eastward, including portions of

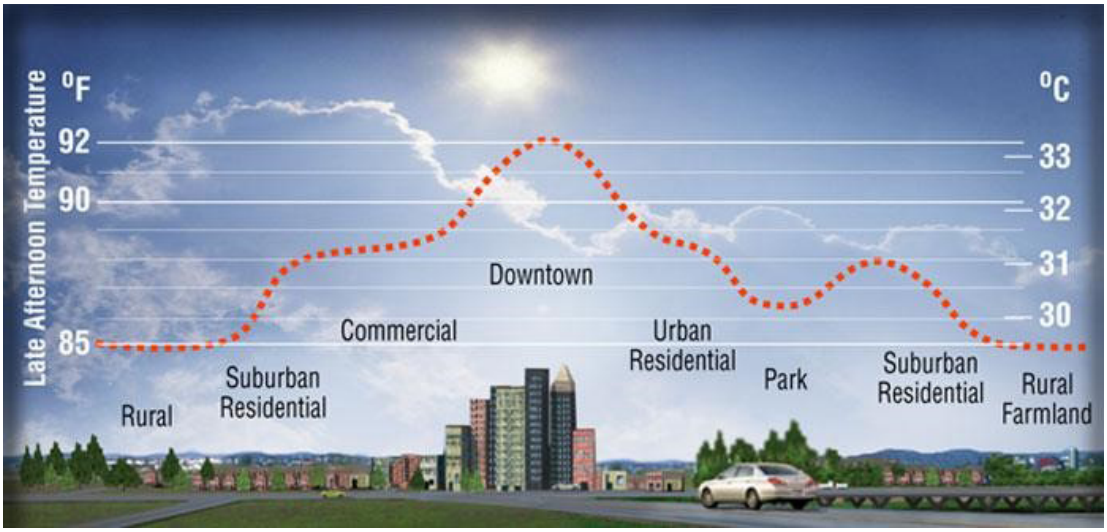


Figure 1. Urban heat island profile showing the elevation in urban air temperature compared with rural air temperature. (Image courtesy of Heat Island Group, Lawrence Berkeley National Laboratory).

Canada (Khan et al., 2020), and isolated populations have been identified in areas in the Western states (Couper & Mordecai, 2022). This urban-dwelling mosquito is capable of reproducing in small containers, such as flowerpots (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 (Ledesma & Harrington, 2015; Morchón et al., 2012; Nelson, 2016).

As mosquito vectors expand their territory and new non-native vectors are introduced (e.g., *Aedes notoscriptus* introduction and establishment within California) (Metzger et al., 2022; Peterson & Campbell, 2015) 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 one that can also sustain sufficient heat to allow maturation of ingested microfilariae into the infective L3 within this intermediate host. It has been shown in three mosquito species that maturation of larvae ceases at temperatures below 57˚F (14˚C) (Christensen & Hollander, 1978; Fortin & Slocombe, 1981). Heartworm transmission does decrease in colder months, but the presence of microclimates in urban areas suggests that the risk of heartworm transmission never reaches zero (Nelson, 2016). Furthermore, some species of mosquitoes overwinter as adults (Bolling et al., 2007; Farajollahi et al., 2005; Hanson & Craig, 1995; Hawley et al., 1989; Hudson, 1978; Romi et al., 2006). While heartworm larval development in these mosquitoes may cease in cool temperatures, development quickly resumes with subsequent warming (Christensen & Hollander, 1978; Ernst & 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 & Lok, 1998). 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 vector, 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 & Navarro-Silva, 2004)
- *Aedes sticticus* (3 months) (Gjullin et al., 1950)
- *Ochlerotatus* (formerly *Aedes*) *trivittatus* (2 months) (Christensen & Rowley, 1978)
- *Aedes vexans* (2 months) (Gjullin et al., 1950)
- *Ochlerotatus* (formerly *Aedes*) *canadensis* (several months) (Pratt & Moore, 1960)
- *Anopheles quadrimaculatus* (4 to 5 months) (Hinman & Hurlbut, 1940).

Survey studies of trapped mosquitoes collected at various locations have demonstrated heartworm infection rates in mosquitoes ranging from 2.1% to 19.4% (Holderman et al., 2021; McKay et al., 2013). 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 74% inside the facilities (McKay et al., 2013). Based upon these data, it is important to protect pets from mosquito exposure (see **Vector Control** starting on page 11) in addition to administering year-round heartworm preventive.

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 improbable.

BIOLOGY AND LIFE CYCLE

KEY POINTS: BIOLOGY AND LIFE CYCLE

- The relatively long life cycle of *D. immitis* (7 to 9 months) requires a reservoir of infection, a vector capable of transmitting infection, and a susceptible host.
- The mosquito, the required vector for transmission of *D. immitis*, becomes infected as she takes a blood meal from a microfilaremic host.
- The *D. immitis* microfilariae mature within the malpighian tubules of the mosquito, developing into larval stage 1 (L1) , then molting into larval stage 2 (L2), and finally molting into the infective third-stage larvae (L3), which are transmitted to the dog when bitten by the mosquito.
- Once the infective L3 enter the dog's body, they molt into fourth-stage larvae (L4).
- A final molt into juvenile/immature adults occurs between days 50 and 70, while they are migrating through the body; and they eventually reach the smallest pulmonary arteries as early as day 67 after transmission.
- Sexual maturity occurs about day 120 post infection with dogs developing patent infections (i.e., having circulating microfilariae) as early as 6 months but usually by 7 to 9 months after infection.
- 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 to the successful management of infected dogs.

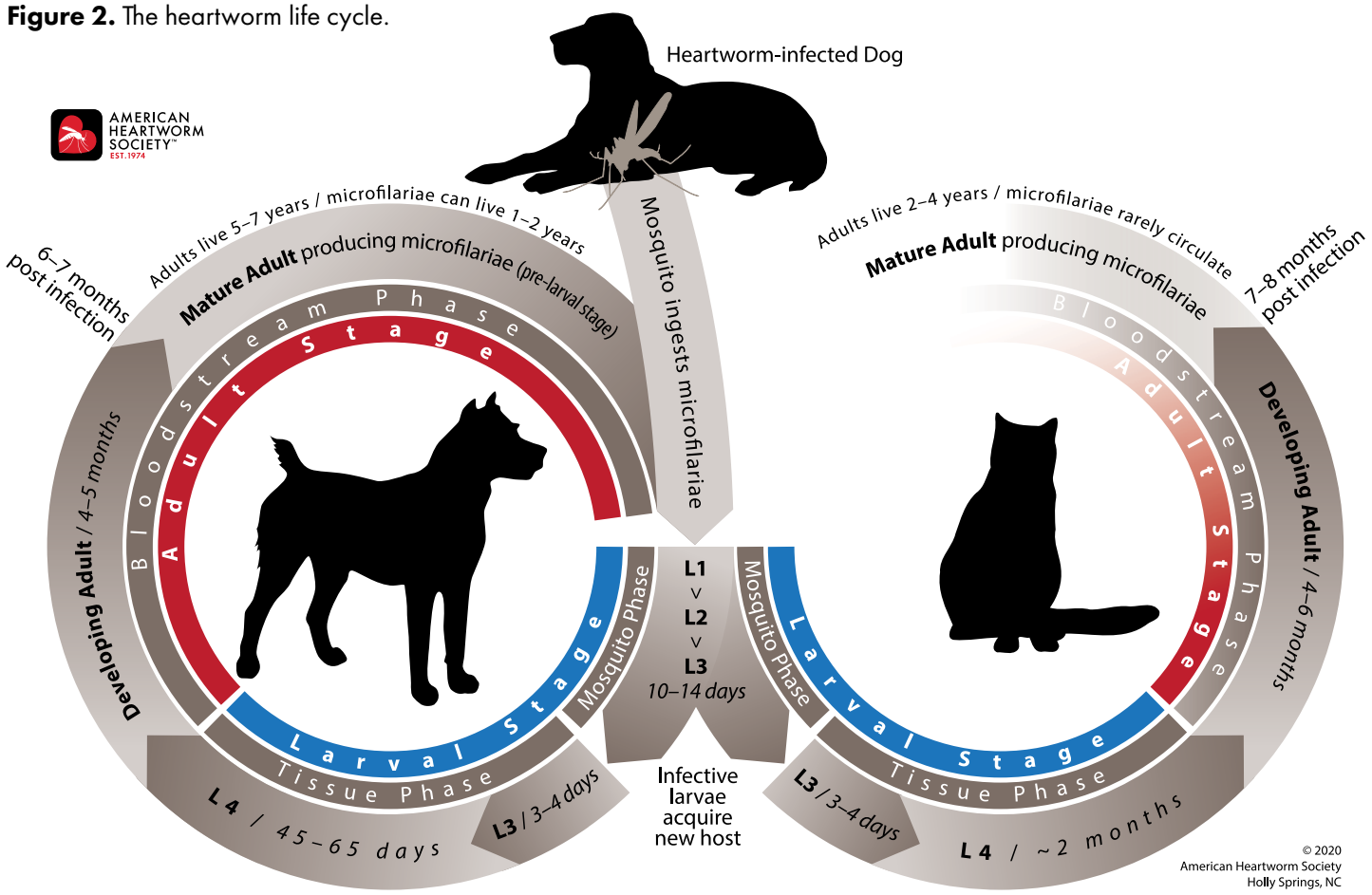
The life cycle of *Dirofilaria immitis* is relatively long (usually 7 to 9 months) compared with most parasitic nematodes (Kotani and Powers, 1982) (**Figure 2**). This protracted life cycle requires a reservoir of infection, a vector capable of transmitting infection, and a susceptible host.

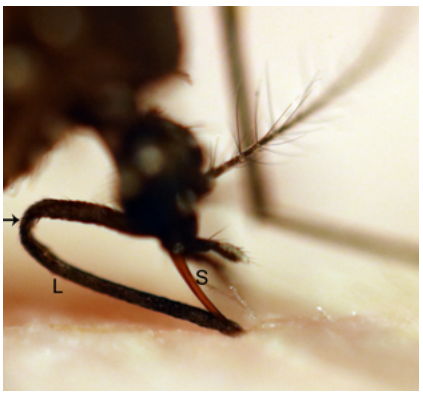
The domestic dog and some wild canids are the normal definitive hosts for heartworms and are inclined to develop high microfilaria counts, thus allowing them to serve as the main reservoir of infection. Less suitable hosts, such as cats and ferrets, occasionally have low-level, transient microfilaremia and theoretically may serve as a limited source of infection for mosquitoes during their short periods of microfilaremia (McCall et al., 2008b).

The mosquito, the required vector for transmission of *D. immitis*, becomes infected as she takes a blood meal from a microfilaremic host. It is important to note that microfilariae cannot develop into adult heartworms without first developing into L1 in the Malpighian tubules of the mosquito, then molting into L2, and finally molting into L3 (Taylor, 1960). The L3, the infective stage, then migrate via the body cavity to the head and mouthparts of the mosquito, where they are positioned for transmission. The time required for the development of microfilariae to the infective stage within the mosquito is temperature dependent. At 27°C (80.6°F) and 80% relative humidity, development takes about 10 to 14 days; with cooler temperatures maturation takes longer (Kartman, 1953; Slocombe et al., 1989).

Once in the mouth parts, transmission of the infective L3 is accomplished when an infected mosquito again takes a blood meal. As the mosquito's stylet penetrates an animal's skin, the labium (lower lip) is forced to fold back at a dramatic angle (**Figure 3**). When this occurs, larvae rupture through the labium causing leakage of hemolymph and infective larvae onto the surface of the host's skin (McGreevy et al., 1974).

Figure 2. The heartworm life cycle.





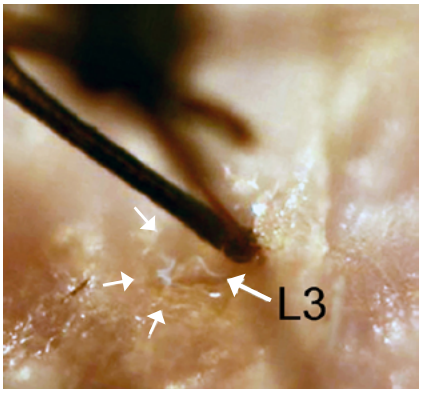


Figure 3. Image of a feeding mosquito (at right, top) indicating how deeply the stylet (S) penetrates the skin and the dramatic folding (*black arrow*) of the labium (L). Magnified image of a feeding mosquito (at right, bottom) indicating the release of a hemolymph pool (*white arrows*) containing infective heartworm larvae (L3). Photographic images courtesy of Stephen Jones, DVM.

Immediately after the blood meal, these L3 enter the animal's body via the puncture wound made by the mosquito. As early as day 3, and by days 9 to 12, the L3 molt into fourth-stage larvae (L4) where they appear to travel between subcutaneous tissues and muscle fibers during early migration within the infected animal. As they continue this migration, a final molt into sexually immature adults occurs between days 50 and 70, where they continue to migrate through the body, enter the circulatory system, and are transported toward the heart and lungs (Kotani & Powers, 1982; Kume & Itagaki, 1955; Lichtenfels et al., 1985; Orihel, 1961). These immature adults reach the pulmonary vasculature as early as day 67 and all have arrived by days 90 to 120.

At the time of arrival in the pulmonary arteries, these immature heartworms measure between 1 and 1.5 inches in length. As they mature, heartworms ultimately grow tenfold to reach 10 to 12 inches in length. Sexual maturity occurs about day 120 post infection with dogs developing patent infections (i.e., having circulating microfilariae) as early as 6 months but usually by 7 to 9 months after infection (Kotani & Powers, 1982; Orihel, 1961). It has been shown that adult worms can live at

least 5 years, if not longer, in infected dogs, and the dog can serve as a reservoir of microfilariae for infecting mosquitoes for at least 7 years (Newton, 1968; Starkey, 2024, personal communication).

When sexually immature adult heartworms first arrive in the lungs the flow of blood forces them into the small pulmonary arteries (Rawlings, 1980). As the worms increase in size, they progressively occupy larger and larger arteries until they become fully mature. The eventual location of the mature adult worm appears to depend mainly on the size of the dog and the worm burden. A medium-sized dog (e.g., 10–15 kg) with a low worm burden (≤5) usually has worms mainly in the lobar arteries and main pulmonary artery. As the worm burden increases, worms also can be found in the right ventricle. Dogs with more than 40 worms are more likely to develop caval syndrome, where the worms maneuver into the right ventricle, right atrium, and the vena cava, thus interfering with valvular function and blood flow and producing hemolysis, liver and kidney dysfunction, and heart failure (Atwell & Buoro, 1988; Ishihara et al., 1978; Jackson, 1974).

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 to be able to effectively select the most appropriate adulticidal treatment option and treatment time, and to convey realistic expectations to the client for the outcome of therapy.

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 to be able to effectively select the most appropriate adulticidal treatment option and treatment time, and to convey realistic expectations to the client for the outcome of therapy.

HEARTWORM PREVENTION

KEY POINTS: HEARTWORM PREVENTION

- The FDA-approved heartworm preventives currently marketed (ivermectin, milbemycin oxime, moxidectin, and selamectin) belong to the macrocyclic lactone (ML) class of drugs.
- Macrocyclic lactones, when given according to label instructions, are highly effective and are among the safest medications used in veterinary medicine.
- It is possible for an animal to become infected while appropriately dosed or because of skipped or delayed administration of just one preventive dose.
- While the vast majority of reported claims of lack of efficacy of MLs can be linked to poor compliance/adherence, ML-resistant heartworms have been documented (see **Figure 4**).
- AHS and the FDA recommend year-round administration of FDA-approved preventive drugs to prevent heartworm infection and enhance compliance/adherence.
- Application of an EPA-approved mosquito repellent/ectoparasiticide has been shown to increase the overall efficacy of a heartworm prevention program by controlling the mosquito vector in laboratory studies. Furthermore, use of an FDA-approved ectoparasiticide product can break the transmission cycle and could aid in reducing mosquito populations.
- In addition, reduction of exposure to mosquitoes through standard environmental control of mosquitoes and their breeding environments, and when possible, reducing outdoor exposure during key mosquito feeding periods is recommended.

Macrocyclic lactones, when given according to label instructions, are highly effective and are among the safest medications used in veterinary medicine.

The prescription and subsequent administration of heartworm preventive medication requires authorization by a licensed veterinarian having a valid relationship with the client and patient. To establish this relationship, heartworm prevention must be discussed with the client. In dogs >7 months of age and/or if records of testing within the past 12 months do not exist, it is necessary to test (antigen and microfilariae) the patient before dispensing or prescribing a preventive product (see PRIMARY DIAGNOSTIC SCREENING). This practice avoids delays in detecting subclinical infections, the potential confusion concerning effectiveness of the prevention program if a pre-existing infection becomes evident after beginning preventive (e.g., preventive initiated during the prepatent period), and inadvertently killing adult worms with MLs in the absence of doxycycline and activity restriction, which would be an unintentional "slow-kill" adulticide treatment. Options for effective prevention include several drugs administered monthly either orally or topically, or parenterally at 6-month or 12-month intervals.

Heartworm disease is preventable despite the dog's inherently high susceptibility. Because all dogs living in heartworm-endemic areas are at risk, preventive medications are a high priority. Puppies must be started on prevention consisting of a ML as early as possible, no later than 8 weeks of age. Some FDA-approved products are available for use in puppies as young as 4 weeks of age. In highly endemic regions the addition of a mosquito repellent/ectoparasiticide is warranted to attain maximal protection against infection. Puppies started on a heartworm preventive after 8 weeks of age must be tested 6 months after the initial dose and annually thereafter.

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

Macrocyclic Lactones

The FDA-approved heartworm preventives currently marketed (ivermectin, milbemycin oxime, moxidectin, and selamectin) belong to the ML class of drugs and likely work in concert with the dog's immune system to kill susceptible larval stages (Campbell, 1989; Carithers, 2017; Moreno et al., 2010; Vatta et al., 2014;). These drugs are labeled to affect L3 and L4, and in some instances, microfilariae. Because their filaricidal effect on precardiac (migrating) larvae can be achieved by brief pulsing at very low doses (e.g., monthly) or continuous release of small amounts over long periods (e.g., 6 or 12 months), 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 ML preventive products are labeled for a 30-day dosing interval. Beyond this interval, efficacy against late L4 declines and is unpredictable, as it appears it is more difficult for the immune system to affect these larger worms (Paul, 1986; Carithers, 2017). Sexually immature adult worms, which can be found as early as 52 days post infection, are even less susceptible to the effects of preventives. As worms age, they require progressively longer-term administration to achieve a high level of protection (McCall, 2005; McCall et al., 2001a). Therefore, continuous, year-round administration of heartworm preventive is a partial safeguard in the event of inadvertent delay or omission of regularly scheduled doses.

P-glycoprotein-deficient dogs (e.g., Collies) that have the MDR 1 (or ABCB1) gene mutation are unusually sensitive to a variety of commonly used veterinary drugs, including some antidepressants, antimicrobial agents, opioids, immunosuppressants, and cardiac drugs (Mealey, 2008). (For more information on drugs that cause problems in dogs with the MDR 1 mutation visit http://vcpl.vetmed.wsu.edu/problem-drugs.) The MLs 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 MLs are either accidentally ingested or overdosed because of human error in dosage calculation (Dey et al., 2017). This practice violates the standards of the Animal Medicinal Drug Use Clarification Act of 1994 (AMDUCA) and is an unjustified extra-label drug use. The FDA-approved preventive dosages of all MLs have been shown to be

safe in all breeds, including P-glycoprotein-deficient dogs.

Macrocyclic lactones can be administered by three routes:

- **Oral administration:** Ivermectin, milbemycin oxime, and moxidectin 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 must be given year-round**, but if seasonal treatment is chosen, administration must 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 to meet label requirements for some products (see Reports of Lack of Efficacy below).
- **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 preventive.
- **Parenteral administration:** A single dose of the slow-release (SR) formulation of subcutaneously injected moxidectin-impregnated lipid microspheres provides continuous protection for either 6 or 12 months in dogs 6 or 12 months of age and older, respectively, with the potential to enhance compliance. Consistent administration is necessary for maximal protection.

Reports of Lack of Efficacy

Lack of efficacy (LOE) of a heartworm preventive product is considered by the FDA's Center for Veterinary Medicine (CVM) to be a treated dog testing positive for heartworm regardless of appropriateness of dosage or administration consistency. Possible reasons for reports of LOE include

- Failure to administer sufficient preventive
- Failure to administer the preventive at the proper time
- Failure of a dog to retain a dose
- Failure of expected absorption of the active ingredient
- Biological variation between hosts in drug metabolism and immune response, and in parasite susceptibility to the drug
- Individual worms within a population are resistant to the activity of MLs.

Thus, the exact cause of a specific reported LOE can be difficult or impossible to determine.

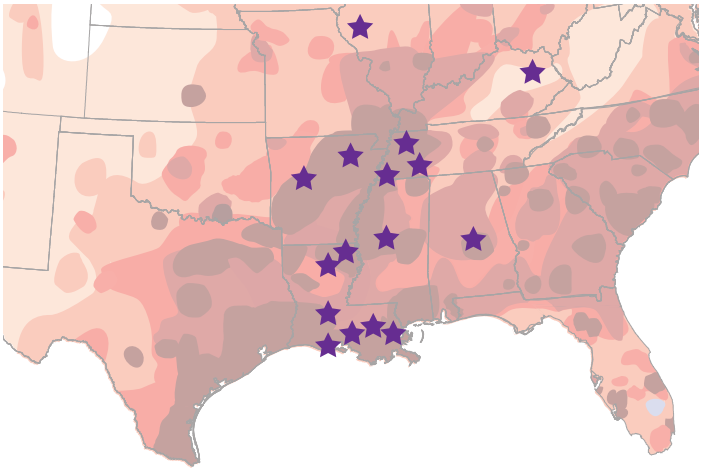
Fortunately, most LOE claims can be explained by compliance/adherence failure, either between the clinic and the client or the client and the pet, rather than product failure. It is possible for an animal to become infected because of skipped or delayed administration of just one preventive dose, particularly 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. In occasional instances, certain products (e.g., topical selamectin in dogs) have been thought to perform sub-optimally due to a potential lack of consistency in absorption and bioavailability in dogs (Sarasola et al., 2002).

It is possible for an animal to become infected because of skipped or delayed administration of just one preventive dose.

Research is ongoing to determine the reasons for LOE. 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 risk and implications of heartworm infection in their area, and that they are providing appropriate year-round heartworm prevention for their pets. The MLs continue to be the only FDA-approved option for preventing heartworm infection and efforts need to be intensified to increase the number of dogs receiving preventive and to increase the number of doses administered per year. Reminder systems must be implemented to assist pet owners in purchasing and administering products in a timely manner.

Macrocylic lactone-resistant heartworms have been identified (**Figure 4**). The extent, the degree of spread, and the reasons for resistance are not well understood and are controversial. Although an algorithm utilizing the microfilarial suppression test (MFST) to help clinicians evaluate cases of suspected resistance to

Figure 4. Known U.S. locations (★) of dogs infected with ML-resistant biotypes of *Dirofilaria immitis*.



MLs was developed (Moorhead et al., 2017; Pulaski et al., 2019), no definitive test for resistance exists, making determination of its distribution difficult. The data suggest that owner compliance/adherence remains the biggest factor in the "failure" of preventives (Atkins et al., 2014; Johnson & Padgett, 2019). Although resistance is concerning, available products remain highly effective and should continue to be used as the manufacturers suggest. A number of experimental studies using commercially-available heartworm preventive products have evaluated product efficacy against known resistant isolates of heartworm (JYD-34 and others) with a single or multiple consecutive doses administered, with moxidectin-containing products providing the highest levels of documented efficacy against resistant isolates of heartworm, however, even with moxidectin, efficacies have ranged from 21.6–100% depending on isolate, dosage, number of doses administered, and timing of dosage in relation to infection day (Bourguinat et al., 2015; McTier et al., 2019; McTier et al., 2021). It must be noted that all ML molecules have been shown to fail to some extent against resistant isolates, and that resistance is a ML class-wide phenomenon.

Vector Control

Heartworm disease has the greatest morbidity and mortality of any vector-borne disease affecting dogs in the United States, and despite the excellent products available to prevent heartworm disease in dogs, the range and number of cases grows annually. Because the mosquito is an obligate intermediate host and vector for heartworms, the opportunity to interrupt the chain of transmission at the level of the vector must not be ignored by the pet owner, the veterinarian, or the local municipalities responsible for environmental health-mosquito abatement. A multimodal approach to address both heartworm transmission and infection needs to be considered as an important opportunity to improve outcomes for both individual dogs and the population at large. There are many examples both in veterinary and human medicine where individual- and community-based multimodal approaches to vector-borne disease control are strongly advocated, if not the standard of care. Examples are Lyme borreliosis in dogs and malaria in humans.

Several tactical approaches can be employed to support the overall strategy of vector control. Vector biology has been addressed elsewhere in these guidelines. The first community-based approach must be elimination of mosquito larval habitats, for example, standing water sources wherever possible or treatment of these habitats with chemical and/or biological tools such as, but not limited to, insect growth regulators, *Bacillus* species, and mosquito fish. Local application

Vector Control Measures to Reduce Heartworm Transmission

- Eliminate sources of standing water where mosquitoes can breed.
- If standing water cannot be eliminated, it should be treated with chemical and/or biological tools such as insect growth regulators, *Bacillus* species, and mosquito fish.
- Utilize local application of insecticidal sprays/fogs and adult mosquito traps.
- Reduce exposure of dogs by limiting outdoor activities during peak mosquito feeding times (dusk and dawn) and avoiding known mosquito habitats.
- Use FDA- or EPA-approved ectoparasiticide products designed for use in dogs:
  - Products with demonstrated insecticidal properties can break the transmission cycle and could aid in reducing mosquito populations.
  - Topical ectoparasiticide products with demonstrated mosquito repellency can reduce and/or block the transmission of infective L3s to animals and microfilariae to mosquito vectors.
  - It is NOT recommended to use DEET on dogs as it is only approved for human use. (Dorman, 1990; Gwaltney-Brant, 2004)

of insecticidal sprays and fogs and deployment of adult mosquito traps are other approaches. Low winds greatly disturb internally directed flight patterns of mosquitoes, and fan-generated wind has been shown to dilute attractants like carbon dioxide and is a practical approach to protecting people and pets in back yard settings (Hoffman & Miller, 2003). Public municipal organizations as well as private professional businesses can provide expert guidance and tools for these efforts.

Direct protective measures that can be recommended to the dog owner include risk-behavior modification such as limiting outdoor activities during peak mosquito feeding times and avoidance of known mosquito habitats. A highly effective direct protective measure is the use of topically applied ectoparasiticide products with demonstrated mosquito repellency and insecticidal claims.

Use of Repellents and Ectoparasiticides

Repellents work by inhibiting blood-feeding by vector mosquitoes and the associated transmission of infective heartworm larvae to the treated dog or microfilariae to mosquitoes. This decreases the likelihood of an uninfected dog becoming infected, or a microfilaremic dog from serving as a reservoir for infecting mosquitoes and subsequently infecting other pets. A permethrin-based repellent was demonstrated to be highly effective (>95%) in preventing mosquito feeding in two well-controlled laboratory studies. When repellent-treated microfilaremic dogs were challenged with uninfected mosquitoes, all of the mosquitoes that showed evidence of feeding died within 3 days (McCall et al., 2017a). Furthermore, the repellent was 95% effective in preventing adult heartworm infection in dogs following exposure to heartworm-infected mosquitoes as compared with the control group (McCall et al., 2017b). These initial study results are encouraging but additional studies are needed to determine outcomes under field conditions.

Ectoparasiticides work by killing mosquitoes that have contacted or fed on a treated animal. Because mosquitoes die within 3 days following exposure to treated dogs, they are incapable of transmitting heartworms. This, in essence, renders the treated dog a non-reservoir, and as an added benefit kills the female mosquito, therefore preventing egg laying, and ultimately reducing the local mosquito population with further reduction in heartworm transmission in the area.

Isoxazolines are widely used on pets for treatment and control of fleas, ticks, mites and other arthropods and are being re-purposed for efficacy against mosquito vectors of *D. immitis* and other diseases of animals and people (Miglianico et al., 2018). Treatment of dogs with systemic isoxazolines should greatly impact the transmission of mosquito-borne diseases by killing adult mosquitoes and preventing, or suppressing, the females' egg production. While treatment of dogs with isoxazolines has not been shown to prevent mosquito biting with transmission of *D. immitis* to the dog, in laboratory studies, most mosquitoes that feed on blood from dogs treated with sarolaner (Geurden et al., 2023), fluralaner (Duncan et al., 2023; Evans et al., 2023) or afoxolaner (Liebenberg et al., 2017) die within 24–48 hours after feeding on blood from treated dogs, and this should limit further direct transmission as L3s will be unable to develop within that period of time; in addition, this can greatly reduce future transmission by reducing egg production by females, and thus, the local population of mosquitoes (Evans et al., 2023; Geurden et al., 2023).

While repellents and ectoparasiticides alone or together are helpful, they are not completely effective as monotherapy for heartworm prevention in highly endemic areas. In a study using a repellent-ectoparasiticide along with a ML, however, dogs challenged with mosquitoes carrying a highly ML-resistant strain of heartworm were 100% protected from infection (McCall et al., 2017b). Thus, a ML preventive, concurrent with the use of a topical mosquito repellent-ectoparasiticide, may provide more complete protection from resistant as well as susceptible heartworms.

Multimodal Risk Management

The risk management approach for heartworm disease in dogs is a process of qualitatively and quantitatively evaluating the threat of infection and disease followed by coordinated and reasonable application of countermeasures to mitigate each of those threats. The threat of heartworm infection can be readily assessed from the AHS Incidence Maps (heartwormsociety.org) and from information provided elsewhere in these Guidelines.

Veterinarians should be encouraged to make recommendations for heartworm infection and disease countermeasures that are commensurate with the known or anticipated level of threat. For example, a dog residing in an area of low incidence may be administered a ML product as a reasonable year-round countermeasure. As the threat increases, the application of a topical ectoparasiticide product having demonstrated mosquito repellency and insecticidal

The risk management approach for heartworm disease in dogs is a process of qualitatively and quantitatively evaluating the threat of infection and disease followed by coordinated and reasonable application of countermeasures to mitigate each of those threats. The threat of heartworm infection can be readily assessed from the AHS Incidence Maps (heartwormsociety.org).

claims during the months of highest mosquito activity is a reasonable addition to the year-round ML. For dogs residing in areas of the country where the threat is highest and sustained, the best recommendation to counter the threat of heartworm infection is year-round use of both a ML and a topical ectoparasiticide product with demonstrated mosquito repellency and insecticidal claims, in addition to ensuring environmental mosquito abatement measures are taken.

Using a multimodal risk-management approach to address the threat of heartworm infection and disease enhances the potential to break the cycle of heartworm transmission, addresses the challenges of resistant phenotypes in the heartworm population, and benefits both the individual dog as well as the population at risk.

PRIMARY DIAGNOSTIC SCREENING

KEY POINTS: PRIMARY DIAGNOSTIC SCREENING

- The American Heartworm Society recommends annual screening for all dogs over 7 months of age with both an antigen and a microfilaria test.
- The current generation of heartworm antigen tests identifies most infections consisting of at least one mature female worm and are nearly 100% specific. Differences in sensitivity exist especially in cases with low worm burdens and/or low antigenemia.
- All positive antigen tests should be confirmed through additional testing prior to the administration of any therapy. Confirmation is accomplished upon the identification of circulating microfilariae, or when another positive result is obtained utilizing a different type of antigen test.
- A no antigen detected (NAD) test result does not confirm that a dog is free of heartworm infection; it simply indicates that no antigen can be detected by that particular testing method.
- **ALL DOGS MUST BE TESTED FOR MICROFILARIAE ANNUALLY!** Microfilaremia validates serologic results, identifies the patient as a potential reservoir of infection, alerts the veterinarian to a high microfilariae burden, and may aid in detecting infected dogs that test falsely negative due to presence of immune complexes.
- Heat treatment of serum samples prior to heartworm antigen tests to release blocked antigen is currently available through reference laboratories. However, the routine heating of blood samples IS NOT PRESENTLY RECOMMENDED for heartworm screening in dogs.
- In cases of noncompliance or changing the brand or type of heartworm preventive, the dog must be antigen and microfilaria tested prior to starting or changing products.

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. Choosing to prescribe and administer heartworm preventive without performing appropriate diagnostic tests to determine a dog's heartworm status is NOT RECOMMENDED. Should the dog be infected with heartworms, that would be initiating a sub-optimal "slow-kill" protocol without consideration for activity restriction or doxycycline and could pose additional safety risks to the dog. Should a situation arise where antigen testing cannot be performed, at a minimum, examine a drop of blood for microfilariae prior to proceeding with preventive administration. Treating without

knowing a dog's true heartworm status, whether due to lack of testing or inappropriate testing, could contribute to the selection for increased numbers of resistant *Dirofilaria immitis* within the population. If all of the ML-susceptible L3s, L4s, and/or microfilariae are eliminated, that will leave only ML-resistant stages to continue maturing and/or to be available for transmission to local mosquitoes. This shifts the local heartworm population toward a larger proportion of resistant individuals. Inclusion of vector-control measures (see page 11) should be implemented.

Test Timing for Optimal Results

Currently available heartworm antigen tests detect protein secreted mainly by adult female *Dirofilaria immitis* (Courtney & Cornell, 1990; Weil, 1987), and the most useful microfilaria tests concentrate microfilariae (modified Knott or filtration test) and allow for greater sensitivity (Georgi & 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 a ML preventive (McCall et al., 2001b). 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 antigen testing a dog prior to 7 months of age, and unless there is a suspicion of transplacental passage or blood transfusion of microfilariae, microfilariae testing prior to 7 months of age would also be unnecessary.

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 (e.g., dogs adopted from shelters). It has come to light that in some dogs infected with heartworms, antigen blocking, from antigen-antibody complexes, may lead to false-negative antigen test results. These dogs will be antigen negative and possibly microfilariae positive. In studies conducted on shelter dogs in the southeastern United States, this occurred at a rate of 5.2–7.7% (DiGangi et al., 2017; Gruntmeir et al., 2020; Velasquez et al., 2014). It is important that these dogs are identified and treated to reduce the local reservoir population and decrease the potential for selection of resistant subpopulations of heartworms. There will be instances where an infected dog is both antigen and microfilaria negative.

Antigen Tests

Enzyme-linked immunosorbent assay (ELISA), immunochromatographic, and immunofluorescent antibody test systems are available for detecting circulating heartworm antigen, a glycoprotein generated by the reproductive tracts of adult worms, predominantly female worms (Weil, 1987; Weil et al., 1985). Each testing format has proven to be clinically useful. The current generation of heartworm antigen tests identifies most infections consisting of at least one mature female worm and are nearly 100% specific (Atkins, 2003; Courtney & Zeng, 2001; Lee et al., 2011; McCall et al., 2001b). Differences in sensitivity exist especially in cases with low worm burdens and/or low antigenemia. Currently there are no verified tests consistently capable of detecting infections of only adult male worms (Gruntmeir et al., 2020). Heat-treatment of samples prior to testing has been reported to increase detection of male-only infections (Gruntmeir et al., 2020). However, dogs experimentally infected via transplantation harboring live male worms only (1, 1, 4, or 6/dog) at necropsy 20.3 months following one month of doxycycline (10 mg/kg q24 hours for 72–98 days) plus prophylactic doses of ivermectin (6 µg/kg every other week for 6–7 doses) were antigen-negative with and without heat treatment of the sample (McCall et al., 2023a).

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 process has been simplified for several test kits that use devices that minimize the number of steps and partially automate the procedure. Both false-positive and false-negative results can occur. When a test result is unexpected, either positive or negative, the test result should be corroborated by using a new sample on a different type of test platform. If the result remains ambiguous, independent confirmation by a reference laboratory is recommended to confirm or refute the result.

While a positive heartworm antigen test indicates the presence of specific heartworm antigen, there are factors that can initiate a false-positive result. Currently, it is recommended that all positive antigen tests be confirmed through additional testing prior to the administration of any therapy including the use of MLs, doxycycline, or melarsomine. Confirmation is accomplished upon the identification of circulating microfilariae, or when a positive result is obtained utilizing a different type of antigen test. Ultrasonographic visualization of adult heartworms within the heart or pulmonary artery is also confirmatory. Thoracic radiography depicting signs of heartworm disease, while not diagnostic of current infection, can be supportive of heartworm disease. In general, it is better to trust rather than reject positive antigen test results.

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, low antigen levels from infections with young adult female worms and/or only a few adult females (Grieve & Knight, 1985; Wang, 1998), and the presence of antigen-antibody complexes which can reduce or completely block antigen detection. Therefore, quantitative analysis of antigen results is highly speculative and requires correlation with other relevant information. The color intensity of a positive antigen test result cannot reliably be used to determine the level of heartworm burden, and the use of antigen testing in this manner should be largely discouraged.

A negative antigen test result does not confirm that an animal is free of heartworm infection; it simply indicates that no antigen can be detected by that particular testing methodology. As such, a negative test result should be interpreted and documented more accurately as no antigen detected (NAD) rather than "negative."

False-negative test results occur most commonly when infections are light, female worms are immature, only male worms are present, and/or the test kit instructions have not been followed. There are also cases of antigen blocking from antigen-antibody complexes interfering with antigen testing, resulting in false-negative tests. Laboratory studies have shown that heating serum will release blocked antigen and result in more positive test results (DiGangi et al., 2017; Gruntmeir et al., 2020; Velasquez et al., 2014). (For more on heat treatment, see the box above.)

Data are limited on heat-treatment antigen testing related to whether it should be used as a screening diagnostic tool for dogs. The available data indicates that heat treatment will likely reveal antigen in a heartworm-infected dog that initially tests false negative (DiGangi et al., 2017; Gruntmeir et al., 2020; Little et al., 2018; Velasquez et al., 2014). At the same time, the risk of generating a false-positive result seems quite low. If the ONLY diagnostic result to deem a dog as heartworm-positive is obtained following heat treatment (meaning NAD on non-heated antigen and no microfilariae are detected with a highly sensitive microfilaria test), further diagnostics are warranted to determine the current overall health status of the patient and to rule out other parasites which may cross-react on the

When Should Heat Treatment of Serum Samples Be Considered?

Heat treatment of serum samples prior to heartworm antigen tests, as well as other non-heat methods to release blocked antigen, is currently available through reference laboratories. This process should be considered when a NAD antigen test result does not correlate with the presence of circulating microfilariae, suspicion due to active clinical disease (e.g., signs, radiographic changes), when initial antigen-based diagnostic results are discordant, or a history of lack of adherent administration of heartworm prevention in a high-risk dog in or from a high-risk area. However, the routine heating of blood samples IS NOT PRESENTLY RECOMMENDED for routine heartworm screening.

While heat treatment of samples has been shown to release blocked antigen that can cause false-negative test results, it is contrary to the label instructions for commonly used in-house tests and may interfere with the accuracy of results of not only heartworm testing but also the results of combination tests that include antibody detection of other infectious agents. Further studies on the possible cross-reactivity of heartworms with other helminths are needed to more accurately interpret the conversion from "NAD" to "antigen positive" after heat treatment.

How to Perform the Modified Knott Test

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 5**) (Rawlings, 1986). The entire sediment volume must be examined and counted to achieve a quantitative result (microfilariae/mL).

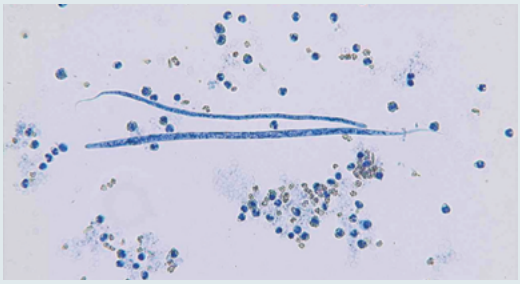


Figure 5. *Acanthocheilonema reconditum* (top) and *Dirofilaria immitis* (below). Photograph courtesy of Byron Blagburn, PhD.

antigen test (e.g. *Dirofilaria repens*, *Angiostrongylus vasorum*, *Spirocerca lupi*) (Aroch et al., 2015; Schnyder & Deplazes, 2012; Sobotyk et al., 2021; Venco et al., 2017). It is our recommendation that monitoring and retesting the patient with antigen and microfilariae tests in 3 months might be the most prudent to determine the most appropriate future diagnostic and/or treatment protocol.

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 ML prevention program (McCall, 2005). Considering this, if a dog is microfilaremic, a positive dog 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.

For more accurate results a concentration technique (modified Knott test) should be used to determine the absence or presence of microfilariae (Georgi & Georgi, 1992; Knott, 1939). The modified Knott test (see box at left) 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*. Dilute formalin (2%) is used as the standard lysing solution for the modified Knott test; however, additional work has revealed that solutions such as acetic acid and distilled water may be suitable options (Evans et al., 2019; Genchi et al., 2021; Long et al., 2020). Should questionable results be obtained, consult a veterinary diagnostic laboratory. For clinics that still have access to a filter apparatus, solutions mentioned above can be used in combination with filter membranes (polycarbonate, 25 mm diameter, 5 µm pore size), most readily available through commercial scientific supply sources.

All dogs should be tested for microfilariae. Microfilaremia validates serologic results, 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 must be antigen and microfilaria tested prior to starting or changing products. A positive test indicates preexisting infection. The dog must always be retested 6 months later (**Figure 6**). A positive test at this time would most likely be due to an infection acquired before starting or resuming preventive therapy; however, in

Figure 6. The testing protocol following known noncompliance includes three tests in the first year, with annual testing thereafter.

rare instances, an existing infection might be missed (i.e., 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, such as radiography, point of care ultrasound, and echocardiography, 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 7**). 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 8**). In the worst cases, the right heart eventually enlarges (Bowman & Atkins, 2009; Calvert & Rawlings, 1988; Rawlings, 1986).

Point of Care Ultrasound (POCUS)

Whereas the 'flash ultrasound exam' is a single view used to quickly answer a binary question (is abdominal effusion present?), POCUS exams use standardized views to ensure a methodical assessment. Indications for POCUS in the dog with heartworm infection include respiratory distress, abdominal distention or ballotable fluid wave, muffled heart or lung sounds, right-sided heart murmur, discolored urine, cyanosis, and syncope. Techniques include the thoracic and abdominal focused assessment with sonography for trauma (TFAST and AFAST) (Boysen & Lisciandro, 2013; Lisciandro & Lisciandro, 2021a) and the veterinary brief lung ultrasound examination (Vet BLUE) (Lisciandro & Lisciandro, 2021b). The Global FAST is a comprehensive exam comprised of 15 views from the AFAST, TFAST, and Vet BLUE exams (Lisciandro & Lisciandro, 2021a). In the patient with heartworm infection, the Global FAST exam is used to diagnose cavitary effusions and intracardiac heartworms. With experience, the clinician can also identify lung pathology (e.g., interstitial

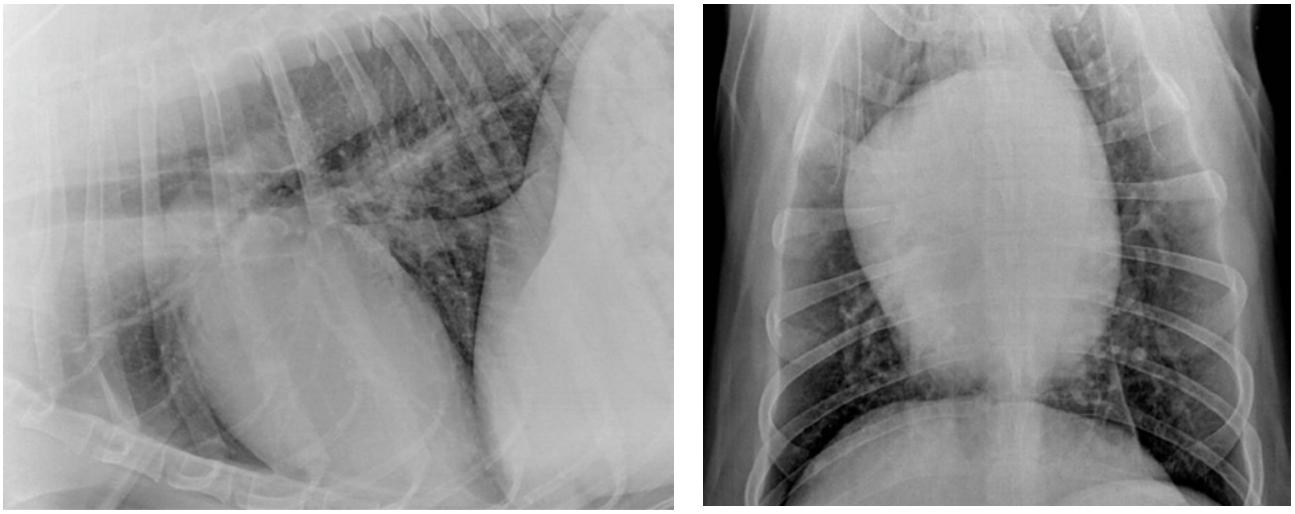


Figure 7. Moderate heartworm disease. Right heart enlargement (reverse "D" shape) is seen in heartworm disease. Radiographic images courtesy of C. Thomas Nelson, DVM.

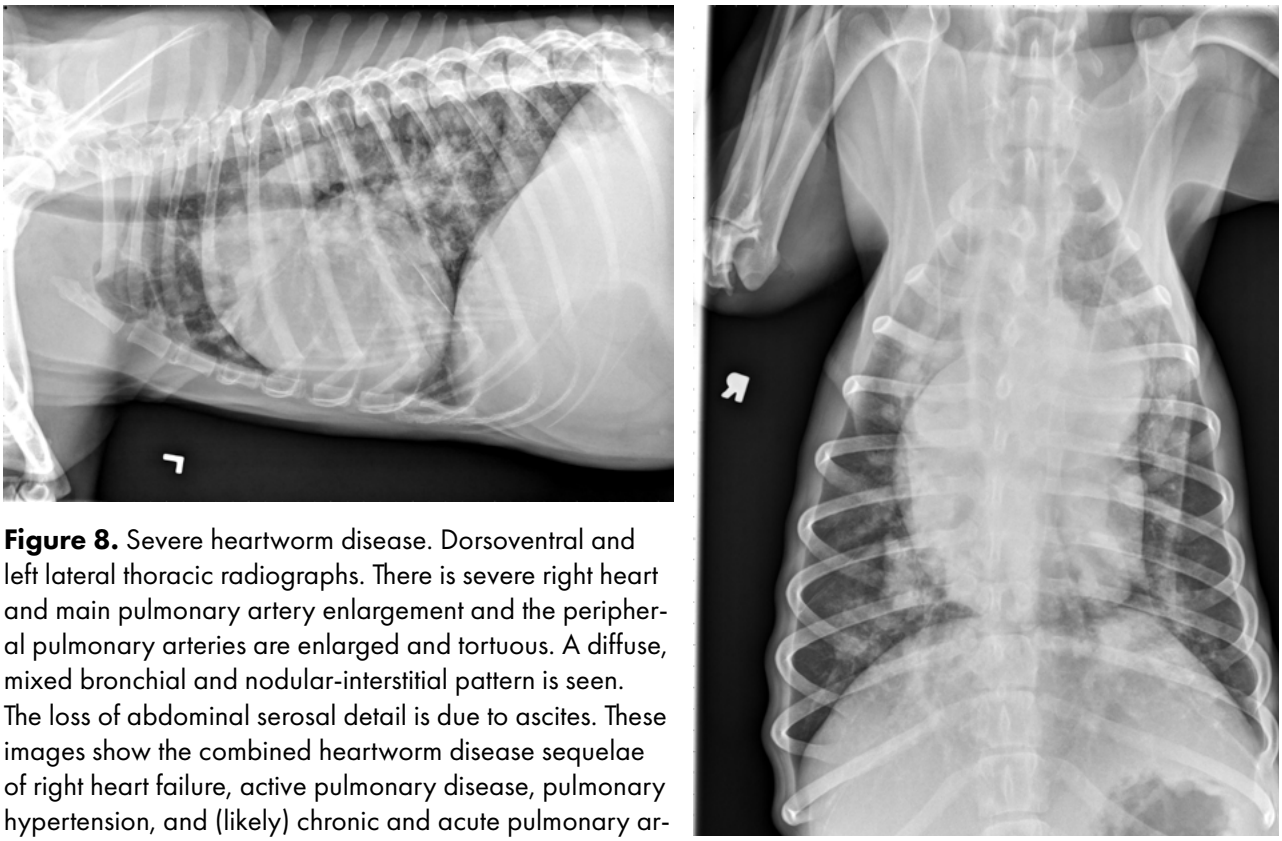


Figure 8. Severe heartworm disease. Dorsoventral and left lateral thoracic radiographs. There is severe right heart and main pulmonary artery enlargement and the peripheral pulmonary arteries are enlarged and tortuous. A diffuse, mixed bronchial and nodular-interstitial pattern is seen. The loss of abdominal serosal detail is due to ascites. These images show the combined heartworm disease sequelae of right heart failure, active pulmonary disease, pulmonary hypertension, and (likely) chronic and acute pulmonary arterial embolism. Radiographic images courtesy of Marisa Ames, DVM.

infiltrates and pulmonary thromboembolism), cardiac changes supportive of pulmonary hypertension, and caudal vena cava and hepatic venous distention (supportive of increased systemic pressures due to right-sided heart dysfunction). 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. The finding of intracardiac worms, anemia, pigmenturia, and signs of low cardiac output (prolonged capillary refill, cool periphery, weak arterial pulses, hypotension) confirms the diagnosis of caval syndrome and urgent heartworm removal is indicated. The POCUS does not replace a thorough physical examination, radiographic evaluation of the pulmonary parenchyma and vasculature, or a complete echocardiogram.

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 and also allows for assessment of cardiac anatomic and functional consequences of the disease (**Figure 9**). It is not an efficient method of making this diagnosis, particularly in larger dogs and dogs with few worms, 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). In a recent retrospective study, 25% of dogs with intracardiac heartworms had clinical evidence of caval syndrome (Romano et al., 2021). Echocardiography is not a definitive diagnostic test for pulmonary hypertension yet helps assess the probability of its presence. Morphologic and functional cardiac and pulmonary vascular changes, the tricuspid regurgitation pressure gradient, and the peak early diastolic pulmonary regurgitation velocity are used to evaluate the probability of pulmonary hypertension (ACVIM Consensus Statement, Reinero et al., 2020). In the

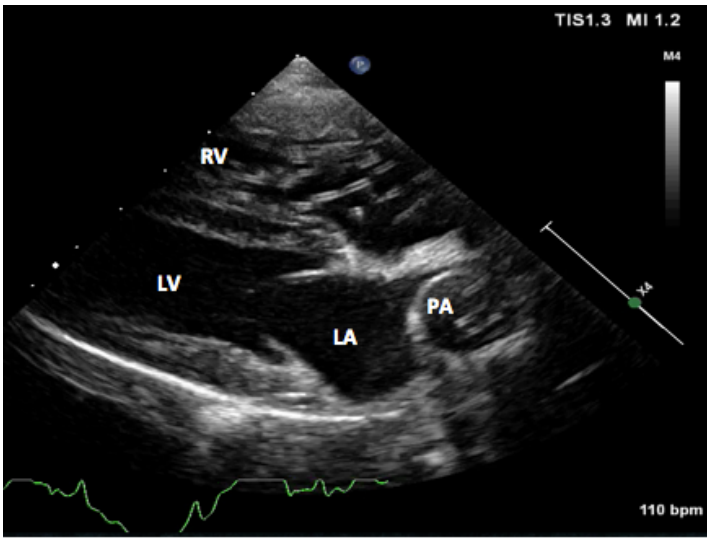


Figure 9. Right parasternal long axis echocardiographic view from an 8-year-old castrated male mixed breed dog that presented for lethargy. There is a large mass of heartworms crossing the tricuspid valve. The right ventricle is severely hypertrophied, and the right atrium is dilated. Worms can also be seen within the severely dilated right branch pulmonary artery. LA, left atrium; LV, left ventricle; PA, right branch pulmonary artery; RV, right ventricle. Image courtesy of Dr. Marisa Ames.

absence of tricuspid or pulmonary regurgitation, the right pulmonary artery distensibility index (Basile et al., 2023; Venco et al., 2014) and pulmonary vein to artery ratio (Matos et al., 2023) are quantitative variables that aid in assessing echocardiographic probability of pulmonary hypertension.

Diagnostics for Pre-Adulticide Evaluation in an Infected Dog

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 key factors, 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), may influence the probability of post-adulticide thromboembolic complications, and the outcome of treatment is not easily measured with standard diagnostic procedures.

Restricting activity is imperative as exercise, excitement, and overheating are harbingers of complications. High activity level of the dog during treatment and for 6 to 8 weeks after the last melarsomine injection is one of the most significant factors contributing to post-adulticidal complications (Dillon et al., 1995a; Fukami et al., 1998). Prior to treatment, the owner's ability and willingness to properly confine treated dogs must be thoroughly investigated. Some helpful resources for pet owners and practitioners are available on the American Heartworm Society website.

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 & Rawlings, 1988; Rawlings, 1986; Rawlings et al., 1981; Romito et al., 2023). 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., 1993a). Regardless of radiographic findings, the protocol to begin elimination of heartworms starting with appropriate heartworm preventive and doxycycline must be initiated as soon as possible.

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 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 potentially 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 must 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.

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.

PRINCIPLES OF HEARTWORM TREATMENT

KEY POINTS: HEARTWORM TREATMENT

- 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 must be stabilized before administering an adulticide. This may require administration of glucocorticosteroids, diuretics, vasodilators, positive inotropic agents, and fluid therapy.
- Melarsomine, administered via deep intramuscular injection into the belly of the epaxial lumbar muscles between the 3rd and 5th lumbar vertebrae, is the only adulticidal drug approved by the FDA.
- Activity restriction during ANY treatment and the subsequent recovery period is ESSENTIAL for minimizing cardiopulmonary complications, regardless of treatment regimen used (i.e., melarsomine versus non-melarsomine). There is a distinct correlation between the activity level of the dog, the severity of disease, and increased risk of treatment-related complications.
- Adjunct therapy with doxycycline for 4 weeks prior to the administration of melarsomine eliminates *Wolbachia*, an endosymbiont bacteria harbored within *D. immitis*, and reduces pathology associated with dead heartworms and disrupts heartworm transmission.
- Administration of doxycycline to a microfilaremic dog does not kill the microfilariae directly, but rather renders the infective larvae later transmitted by infected mosquitoes to other dogs incapable of development to the adult stage, thus reducing the further spread of heartworm disease.
- A macrocyclic lactone preventive must be administered for 2 months prior to administering melarsomine to reduce new infections and eliminate existing susceptible larvae.
- The effectiveness of the macrocyclic lactone can also be potentiated with concurrent use of doxycycline for 4 weeks, as this will essentially eliminate all developing larvae during the first 60 days of treatment.
- Caval syndrome, which develops acutely in some heavily infected dogs when adult heartworms partially obstruct blood flow through the tricuspid valve, is usually fatal within 2 days if surgical extraction of the worms is not pursued promptly.
- The American Heartworm Society's recommended heartworm management protocol is outlined in detail in Table 2 on page 26.

Table 1. Summary of Clinical Signs of Canine Heartworm Disease

Mild (Class 1)	Asymptomatic or cough
Moderate (Class 2	Cough, activity intolerance, abnormal lung sounds
Severe (Class 3)	Cough, activity 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 (Class 4)	Sudden onset of severe lethargy and weakness accompanied by hemoglobinemia and hemoglobinuria

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, young adults, and adults) with minimal post-treatment complications. Dogs exhibiting severe clinical signs of heartworm disease must be stabilized before administering an adulticide. This may require administration of glucocorticosteroids, diuretics (e.g. furosemide), pulmonary arterial vasodilators (e.g. sildenafil), positive inotropic agents (e.g. pimobendan), and fluid therapy.

A thorough understanding of the host–parasite relationship is necessary to effectively manage all cases. As expected, the number of worms affects 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 activity-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., 1995a). 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).

Heartworms cause extensive pathological changes to the entire pulmonary arterial system. Live heartworms in the main pulmonary arteries, lobar arteries, and their branches traumatize the endothelial lining of the arteries as the heartworms are constantly moved back and forth by blood flow. This leads to rugus endarteritis, muscular hypertrophy of arterial walls, fibrosis and overall decreased elasticity of the vessel resulting in increased vascular resistance (**Figure 10**). As worms die from either natural causes or as a result of adulticidal therapy, they collapse and are forced by the blood flow into the segmental branches of the lobar arteries. These dead worms, along with the elicited inflammation,

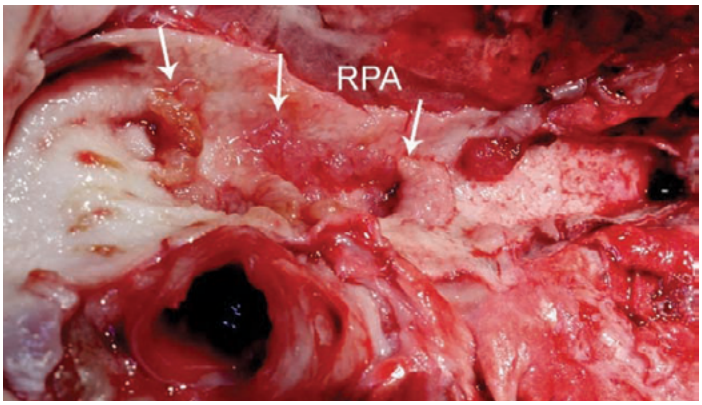


Figure 10. Image of the main trunk of the right pulmonary artery (RPA) exhibiting significant endothelial proliferation (*white arrows*). Photograph courtesy of Stephen Jones, DVM.

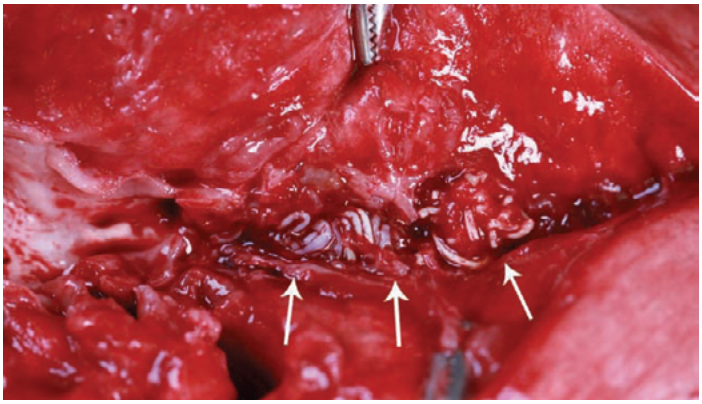


Figure 11. Image of a dead adult heartworm (*white arrows*) lodged in a distal pulmonary artery. Photograph courtesy of Stephen Jones, DVM.

platelet aggregation, and fibrin deposition, result in thromboembolic disease causing even more fibrosis and increased vascular resistance (**Figure 11**). Inflammatory mediators are then carried downstream to the capillary beds in the alveoli leading to edema of Type 1 alveolar cells making them extremely fragile and subject to rupture. During periods of increased activity or exercise, the increased blood flow and/or air flow can lead to rupture of the alveolar cells and their associated capillary. This will cause the alveolus to flood with blood, fibrin to be deposited, and even more fibrosis (Case et al., 1995; Dillon et al., 1995b; Hoskins et al., 1985; Rawlings et al., 1993b). As blood flow becomes restricted, pulmonary artery pressures can increase. In severe cases, right-sided heart failure can ensue, or in a worst-case scenario, retrograde movement of heartworms into the right ventricle, potentially leading to the development of caval syndrome.

Adulticide Therapy

Melarsomine Dihydrochloride

Melarsomine, administered via deep intramuscular injection into the belly of the epaxial lumbar muscles between the 3rd and 5th lumbar vertebrae, 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. Administration of an analgesic such as tramadol, gabapentin, or hydrocodone at the time of injection reduces the acute myalgia associated with melarsomine. Activity restriction during the recovery period is ESSENTIAL for minimizing cardiopulmonary complications (see Pulmonary Thromboembolism at right).

Melarsomine has been shown to have activity against immature worms 2 and 4 months old (Dzimianski et al., 1989; Dzimianski et al., 1990; McCall et al., 2010); however, activity against 3-, 5-, and 7-month-old worms has not been assessed. The two-injection protocol with melarsomine (i.e., 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 99% 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, which must be administered on consecutive days.

Staging of the disease, as described on the melarsomine label, 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 (PTE) is an inevitable consequence of successful adulticide therapy and may be severe if infection is heavy and pulmonary arterial disease is extensive. It should be noted that a PTE in the context of a dog infected with heartworms differs from PTEs in humans. In humans, PTEs result from clot formation and embolization (Tarbox & Swaroop, 2013); in dogs with heartworms, PTEs are comprised of components of the worms themselves which lead to platelet aggregation and subsequent fibrin deposition (Atwell & Tarish, 1995; Sutton, 1988). 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 early as 5 days and as late as 4 weeks after adulticide administration (Hirano et al., 1992; Personal communications, 2024, C. Thomas Nelson & John McCall). Mild embolism in relatively healthy areas of lung may not be clinically apparent but are still leading to irreversible lung damage. A pivotal factor in reducing the risk of thromboembolic complications is STRICT activity restriction. Pre-emptive administration of glucocorticosteroids will reduce the inflammation associated with the foreign proteins released from dead heartworms as well as the effects of inflammatory mediators on Type 1 alveolar cells resulting in less fibrosis and permanent lung damage (Atwell & Tarish, 1995).

Adjunct Therapy

Steroids

Administration of diminishing anti-inflammatory doses of glucocorticosteroids helps control clinical signs of PTE (Atwell & Tarish, 1995). The efficacy of melarsomine when used in conjunction with prednisone (Dzimianski et al., 2010) is not decreased; therefore, the use of glucocorticosteroids is recommended. It should be noted that the implementation of steroids prior to melarsomine treatment is ONLY recommended if the dog is already exhibiting clinical signs of heartworm disease or is microfilaremic and being administered a microfilaricidal dose of a ML. However, all dogs should be administered tapering doses of glucocorticosteroids in conjunction with melarsomine injections. Administration of glucocorticosteroids will reduce the inflammation associated with the foreign proteins released from dead heartworms as well as the effects of inflammatory mediators on Type 1 alveolar cells resulting in less fibrosis and permanent lung damage (Atwell & Tarish, 1995).

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, endosymbiotic 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 L3 and L4 heartworm larvae and about 70% effective against sexually immature heartworms present between days 65 and 94 (McCall et al., 2011). Administration of doxycycline (or doxycycline plus ivermectin) to a microfilaremic dog does not kill the microfilariae directly, but rather renders the infective larvae transmitted later by infected mosquitoes to other dogs incapable of development to the adult stage, presumably by killing the microfilariae's life-supporting *Wolbachia* endosymbiont. In several studies, microfilariae ingested by mosquitoes that fed on blood from dogs treated with doxycycline developed into infective L3 that appeared to be normal in appearance and motility but were not able to develop into adult worms in a new host, thus blocking transmission of susceptible and resistant biotypes and reducing the risk of selecting for resistant subpopulations of heartworms (McCall et al., 2008a, 2014a, 2023b).

In addition, in dogs with adult infections, doxycycline gradually suppressed microfilaremia (Bazzocchi et al., 2008; McCall et al., 2008a). 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 that the presence of *Wolbachia*, or at least very low numbers of the organism, is necessary for embryogenesis. In *D. immitis* (adults and microfilariae), *Wolbachia* numbers remain low for at least 12 months following doxycycline administration (Rossi et al., 2010).

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., 2005a). Studies have shown that a major *Wolbachia* surface protein (WSP) induces a specific IgG response in hosts infected by *D. immitis* (Kramer et al., 2005b). It is hypothesized that *Wolbachia* may contribute to pulmonary and renal inflammation through its WSP.

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. Doxycycline is administered at 10 mg/kg BID for 4 weeks (Bandi et al., 1999; Kramer et al., 2011; Nelson et al., 2017). A one-month wait period after administration of doxycycline, but before administration of melarsomine is currently recommended, as it does not result in increased pulmonary pathology or worm biomass (Moorhead et al., 2023).

Side effects have been associated with the administration of doxycycline at the recommended dose of 10 mg/kg BID. The most common are GI signs, such as vomiting and diarrhea. If these occur, administering the drug with food may ameliorate these signs. However, if the signs still are present, a reduction in dose may be required, as low as 5 mg/kg BID. Discontinue administration of doxycycline until GI signs abate and the dog is eating. Resume at 7.5 mg/kg BID. If GI signs return try decreasing to 5 mg/kg BID. There are some dogs that cannot tolerate doxycycline and have idiosyncratic reactions. In such cases rifampicin (suggested doses: 10 mg/kg SID or 5 mg/kg BID), which has shown anti-*Wolbachia* activity against other

filarial nematodes related to *Dirofilaria immitis*, may be considered as a last resort instead of doxycycline. Keep in mind that adverse drug interactions may occur between rifampicin and other drugs included in the heartworm-treatment protocol. Alternatively, one could proceed with melarsomine injections after the dog has returned to clinical normalcy (Nelson, 2023). Studies have reported up to 40% of dogs receiving doxycycline developing elevated liver enzymes that returned to normal after discontinuing the drug and the increase did not appear to be clinically significant (Savadelis et al., 2020; Schultz, 2011). In a recent study comparing different doses of doxycycline, dogs that showed elevated liver enzymes either had elevated or high-normal enzymes before doxycycline therapy was initiated. None of the dogs that had elevated enzymes had any demonstrable liver pathology on histopathologic exam (Moorhead et al., 2023)

Minocycline has been shown to be highly effective in eliminating *Wolbachia* organisms from the filarial nematode *Onchocerca gutturosa* (Townson et al., 2006). In a published study conducted with minocycline in *D. immitis*, *Wolbachia* DNA levels in microfilariae from dogs administered minocycline were shown to be decreased at similar levels as doxycycline with a similar side effect profile as is seen with doxycycline (Savadelis et al., 2018). In dogs receiving 10 mg/kg BID PO doxycycline, however, no *Wolbachia* DNA was detected, whereas there were still some dogs that remained positive for *Wolbachia* DNA in the other groups. The recommended dosing regimen is the same as doxycycline, and minocycline is a viable alternative if doxycycline is not available.

Doxycycline has other beneficial attributes besides reducing or eliminating *Wolbachia*. It has immunomodulatory activity through inhibition of various inflammatory pathways and has been shown to ameliorate pulmonary inflammation in a murine sepsis model (Patel et al., 2020). Doxycycline in high concentrations also inhibits protein synthesis in mammalian cells which may aid in the reduction of fibrosis (Budde & McCluskey, 2023).

The use of doxycycline, minocycline, or rifampicin for oral administration to a dog for these purposes is considered extra-label drug use and is permitted under AMDUCA. Keep in mind the importance of antimicrobial stewardship as it relates to using antibiotics as adjunct therapeutics when treating heartworm.

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 potential incomplete efficacy of melarsomine against young adult worms could present a problem in achieving the goal of eliminating all of the worms.

A ML preventive should be administered for 2 months prior to administering melarsomine to reduce new infections and eliminate existing susceptible larvae. The effectiveness of the ML can also be potentiated with concurrent use of doxycycline for 4 weeks, as this will essentially eliminate all developing larvae during the first 60 days of treatment (McCall et al., 2008a; McCall et al., 2011).

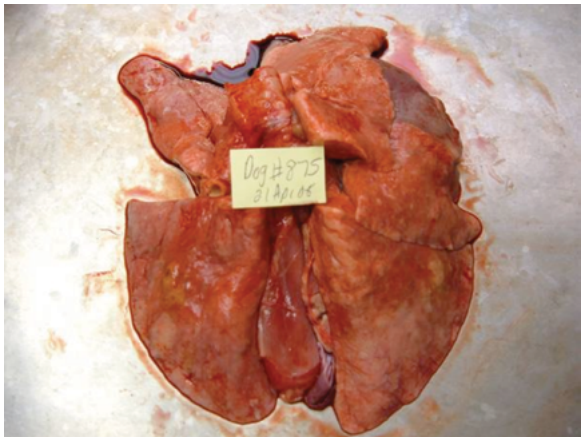
Macrocyclic lactones administered as microfilaricides may cause a rapid decrease in the numbers of microfilariae and must be used with caution in dogs with high microfilarial counts. Pretreatment with antihistamines and glucocorticosteroids will minimize potential reactions. Topical moxidectin is the only FDA-approved heartworm preventive for the treatment of circulating 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., 2014b).

Pretreatment with antihistamines and glucocorticosteroids is advisable in the face of high microfilaria burdens to minimize potential reactions and the dog should be observed for the day after administration of a microfilaricide.

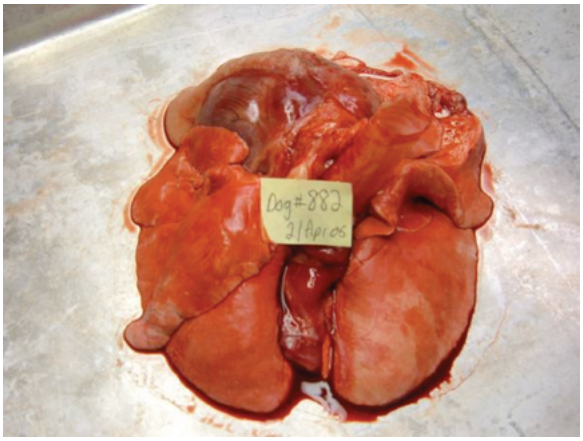
Macrocyclic Lactones/Doxycycline

Studies have shown that experimentally infected heartworm-positive dogs pretreated with ivermectin and doxycycline prior to receiving melarsomine injections had less pulmonary pathology associated with the death of the heartworms (**Figure 12**) (Kramer et al., 2011; McCall et al., 2008a). Necropsy-based studies have not been conducted evaluating other ML-based heartworm preventives + doxycycline followed by a melarsomine regimen.

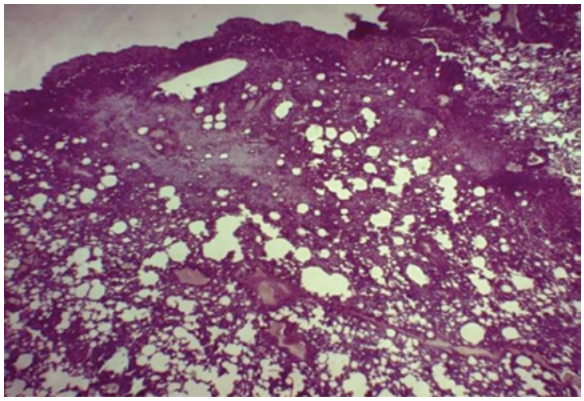
Select MLs (ivermectin and moxidectin) coupled with doxycycline individually suppress embryogenesis and cause morbidity and/or mortality to adult worms (Ames et al., 2020; Grandi et al., 2010; McCall et al., 2008a, 2008b, 2023a; Moorhead et al., 2023; Savadelis



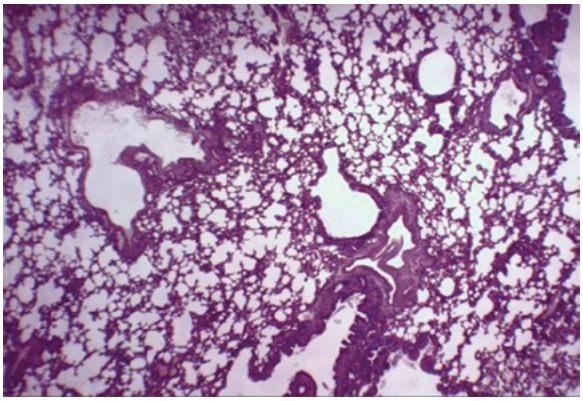
Melarsomine only



Ivermectin / Doxycycline / Melarsomine



Melarsomine only



Ivermectin / Doxycycline / Melarsomine

Figure 12. 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.

et al., 2017). Furthermore, when comparing sizes of adult worms between dogs treated with ivermectin and doxycycline versus dogs not administered either drug, worms weighed less (although not statistically significant) between days 30 and 60 in the dogs receiving the drugs (Moorhead et al., 2023). 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 and effective microfilaricidal and adulticidal activity than ivermectin alone as well as reducing *Wolbachia* numbers more effectively than doxycycline alone (Bazzocchi et al., 2008; McCall et al., 2008a, 2023a). No studies have been published on combining milbemycin oxime or selamectin with doxycycline.

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**; see also the AHS Heartworm Toolkit).

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 et al., 2017). A study on experimentally infected dogs showed that dogs that received doxycycline and ivermectin prior to melarsomine administration had less severe arterial lesions and the virtual absence of thrombi (Kramer et al., 2011).

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 and the dog must be observed for the night and day after administration of a microfilaricide

Table 2. AHS-Recommended Heartworm Management Protocol

Day	Treatment
0	**In a dog diagnosed and verified as heartworm positive, either by:** • Positive antigen (Ag) test verified with microfilaria (MF) test, OR if no MF are detected, • Confirm with a second Ag test with a new sample on a different type of testing platform 1. Administer appropriate heartworm preventive (monthly [topical or oral] or injectable) – If MF are detected, pre-treat with antihistamine and glucocorticosteroids, if not already on prednisone, to reduce risk of anaphylaxis – Observe for at least 8 hours for signs of reaction 2. Administer doxycycline 10 mg/kg BID for 28 consecutive days • Reduces pathology associated with dead heartworms • Disrupts heartworm transmission 3. Begin activity restriction—the more pronounced the signs, the more rigid the activity restriction 4. Administer an EPA- or FDA-approved ectoparasiticide product designed for use in dogs that has demonstrated mosquito-killing activity **If the dog is symptomatic in addition to the items above:** • Stabilize with appropriate therapy and nursing care • Prednisone prescribed at a tapering dose of 0.5 mg/kg BID 1st week, 0.5 mg/kg SID 2nd week, 0.5 mg/kg every other day (EOD) for the 3rd and 4th weeks
30	**Communicate with the client to ensure:** 1. Completion of the full course of doxycycline 2. Administration of heartworm preventive (unless injectable heartworm preventive was administered on day 0) 3. Administration of an EPA- or FDA-approved ectoparasiticide product designed for use in dogs that has demonstrated mosquito-killing activity
31–60	A one-month wait period after administration of doxycycline but before administration of melarsomine is currently recommended
61	1. Administer heartworm preventive (unless injectable heartworm preventive was administered on day 0) 2. Administer 1st (of 3) melarsomine injections, 2.5 mg/kg intramuscularly (IM) a. Monitor for post-injection anaphylaxis b. Prescribe appropriate pain control 3. Prescribe a tapering dose of prednisone of 0.5 mg/kg BID 1st week, 0.5 mg/kg SID 2nd week, 0.5 mg/kg EOD for the 3rd and 4th weeks 4. Start rigid activity restriction (or maintain if started on day 0): cage restriction, on-leash when taken outside to eliminate 5. Administer an EPA- or FDA-approved ectoparasiticide product designed for use in dogs that has demonstrated mosquito-killing activity
90	1. Administer heartworm preventive (unless injectable heartworm preventive was administered on day 0) 2. Administer 2nd (of 3) melarsomine injection, 2.5 mg/kg intramuscularly (IM) a. Monitor for post-injection anaphylaxis b. Prescribe appropriate pain control 3. Prescribe a tapering dose of prednisone of 0.5 mg/kg BID 1st week, 0.5 mg/kg SID 2nd week, 0.5 mg/kg every other day (EOD) for the 3rd and 4th weeks 4. Administer an EPA- or FDA-approved ectoparasiticide product designed for use in dogs that has demonstrated mosquito-killing activity

Table 2 continued on page 27

Table 2. AHS-Recommended Heartworm Management Protocol *(continued from previous page)*

Day	Treatment
91	1. Administer 3rd (of 3) melarsomine injection into the opposite epaxial muscle from the injection site on day 90, 2.5 mg/kg intramuscularly (IM) a. Monitor for post-injection anaphylaxis b. Prescribe appropriate pain control 2. Continue rigid activity restriction for the next 6–8 weeks: cage restriction, on-leash when taken outside to eliminate
120	1. Quantitatively test (e.g., Modified Knott Test) for presence of MF regardless of patient's MF-status on day 0 • If positive, treat with a microfilaricide and retest every 4 weeks until no MF detected. If MF persist, additional testing for resistance should be considered 2. Continue a year-round heartworm and mosquito prevention program as described under Prevention 3. Gradual return to normal activity over the next 4 weeks
365	Resume annual HW-screening protocol (9 months after last melarsomine injection) • Antigen test • Microfilaria test If still Ag-positive, re-treat with 28 days of doxycycline followed by 2 injections (2.5 mg/kg IM each) of melarsomine 24 hours apart • Monitor for post-injection anaphylaxis • Prescribe appropriate pain control • Prescribe a tapering dose of prednisone of 0.5 mg/kg BID 1st week, 0.5 mg/kg SID 2nd week, 0.5 mg/kg for the 3rd and 4th weeks • Institute and maintain strict activity restriction for 6–8 weeks: cage restriction, on-leash when using yard

Managing Interruptions in Scheduled Treatment

Situations may arise where there is an interruption in a scheduled treatment. If the clinic environment necessitates that a delay occurs in any stage of treatment, practitioners may question 1) how to resume the treatment protocol; and 2) whether the protocol itself should be re-started. The following recommendations address these potential questions with the dearth of scientific data that is currently available.

Scenario A: A dog diagnosed and confirmed to have heartworms has been started on heartworm preventive and prescribed doxycycline; however, administration of the first melarsomine injection must be delayed. How long can the practitioner wait before administering melarsomine without having to repeat doxycycline?

Answer: If doxycycline was administered at a dose of at least 5 mg/kg BID for 4 weeks, **and consistent heartworm prevention was administered throughout the elapsed time frame**, there is no need to repeat doxycycline until 6 months has passed.

Scenario B: A dog has been pre-treated with a monthly heartworm preventive and doxycycline and received the first melarsomine injection, but the second and third melarsomine injections are delayed. How long can the practitioner wait before administering the second and third injections?

Answer: In such cases, practitioners can delay the second and third injections for up to 6 months. However, the second and third injections MUST be given within a 24-hour period when the adulticide treatment is resumed. **Again, the dog must be maintained on a preventive without interruption**.

(Bowman & Atkins, 2009). Topical moxidectin is approved by the FDA to eliminate microfilariae (McCall et al., 2014a). 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 (Knight, 1995; McCall et al., 2008b). Current protocols utilizing doxycycline in combination with regular preventive doses of MLs have essentially eliminated the need for post-adulticidal elimination of microfilariae (Bazzocchi et al., 2008; McCall et al., 2008a) although microfilaria testing is still recommended one month after the final melarsomine injection (day 120). Administration of a ML 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 treatment, a microfilaria test should be performed in adulticide-treated dogs at the time the antigen test is conducted 9 months post treatment to coincide with annual heartworm screening recommendations. 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 PREVENTION).

Surgical Extraction of Adult Heartworms

Intracardiac Heartworms and Caval Syndrome (*Dirofilarial Hemoglobinuria*)

Increased pulmonary artery pressures (pulmonary hypertension), decreased cardiac output, relatively large worm burden, simultaneous maturation, and adulticide therapy are purported causes of retrograde migration of heartworm into the right ventricle and right atrium. Adult heartworms partially obstruct blood flow through the tricuspid valve and interfere with valve closure (**Figure 13**). Caval syndrome is diagnosed by the constellation of hemoglobinuria, anemia, and clinical signs of reduced cardiac output (lethargy, weakness, right-sided congestive heart failure) (Atwell and Buoro, 1988; Kitagawa et al., 1986; Venco, 1993). In one recent study, 25% of dogs with intracardiac heartworm were diagnosed with caval syndrome (Romano et al., 2021). The presence of a right-sided systolic heart murmur

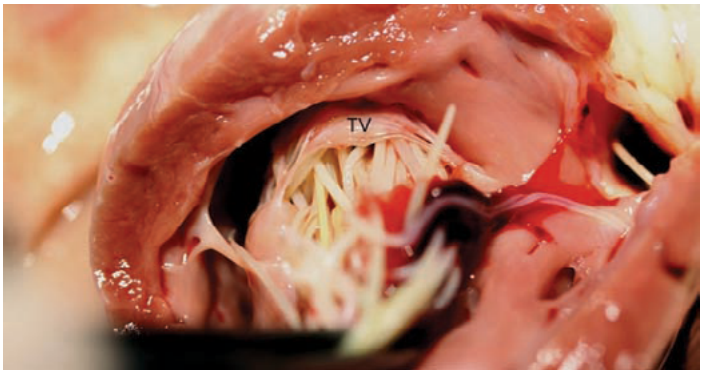


Figure 13. Image of a heart from a dog suffering from caval syndrome as viewed from the right ventricle toward the tricuspid valve (TV). A mass of heartworms completely occludes the valve preventing it from closing. Photograph courtesy of Stephen Jones, DVM.

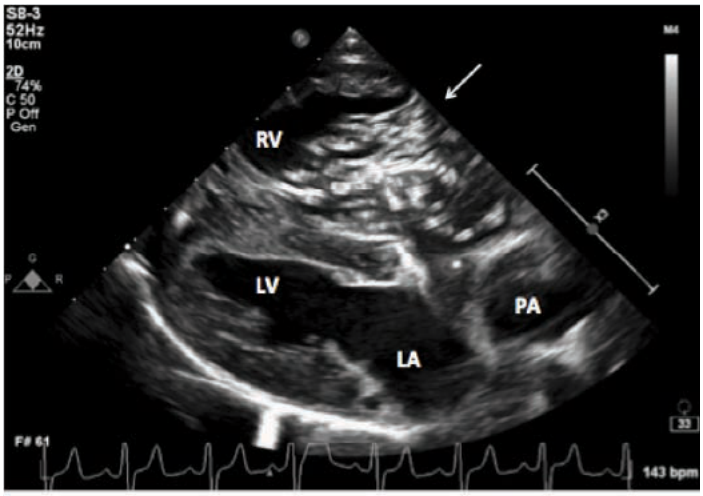


Figure 14. Right parasternal long-axis echocardiogram. There is a large mass of heartworms crossing the tricuspid valve (*arrow*). The right ventricle is hypertrophied and severely dilated. The right atrium and pulmonary artery are also dilated. LA, left atrium; LV, left ventricle; PA, pulmonary artery; RV, right ventricle. Image courtesy of Lauren Markovic, DVM.

is highly supportive of intracardiac heartworms. Intracardiac heartworms can be confirmed by point of care ultrasound and visualization of heartworms within the right atrium, tricuspid orifice, and ventricle, and/or vena cavae (**Figure 14**) (Atkins et al., 1988). Caval syndrome is an emergency and the clinical course usually ends fatally if surgical extraction of the worms is not pursued imminently (Jones, 2016).

Surgical removal of worms from the right atrium and orifice of the tricuspid valve can be accomplished using light sedation (may not be necessary), local anesthesia, and either a rigid or flexible alligator forceps or flexible intravascular retrieval forceps or snare introduced preferentially via the right external jugular vein (Yoon et al., 2013). With fluoroscopic guidance if available, the

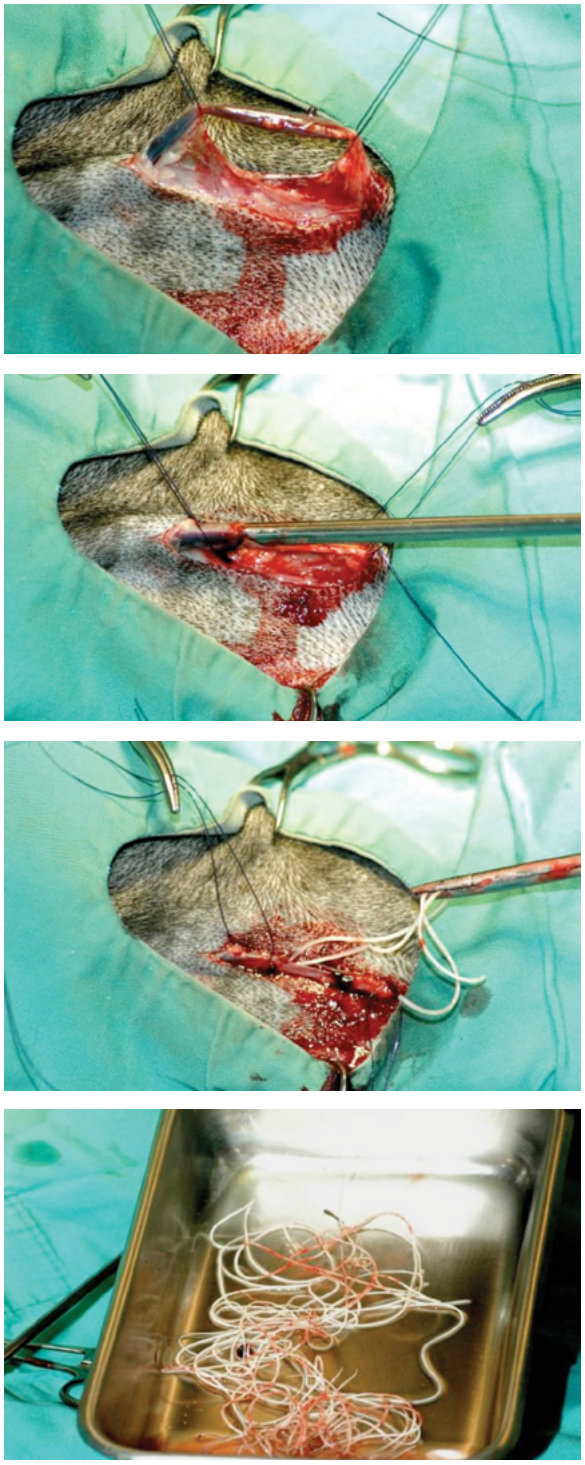


Figure 15. Surgical removal of worms. Photographs courtesy of C. Thomas Nelson, DVM.

instrument should continue to be passed until worms can no longer be retrieved for five consecutive passes (**Figure 15**) (Ishihara et al., 1988; Jackson et al., 1977; Kim et al., 2023). If worms were visualized within the main pulmonary artery on the pre-procedural or intra-procedural ultrasound, flexible forceps, which are often 'steerable,' can be carefully directed across the pulmonary valve and into the main pulmonary artery.

Concurrent ultrasound will help confirm the successful removal of intracardiac worms. 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. Immediately following recovery from surgery, initiation of the AHS-recommended treatment protocol by starting doxycycline and appropriate heartworm preventive with subsequent melarsomine injections is recommended to eliminate remaining worms. Dogs with high intracardiac worm burdens (e.g., worms filling the right atrium) yet without caval syndrome will likely benefit from transvenous extraction. Dogs with only a few worms visualized within the right chambers are usually treated with medical therapy (e.g., sildenafil and pimobendan) immediately followed by the AHS-recommended treatment protocol without the need for transvenous extraction (Pariaut et al., 2020; Tjostheim et al., 2019; Vila & Alost, 2023).

Spectrum of Care Principles that May Be Incorporated into Heartworm Treatment Protocols

NOTE: The following considerations do not supersede the AHS-recommended three-injection protocol. These are sub-optimal strategies to remove adult heartworms when treating with the recommended protocol is not possible.

When the recommended three-injection protocol is not possible, there are a variety of options for treatment. Please note that adverse events and complication rates have been described (if not stated below, data was not available). It should be noted that the long-term safety and side effects following completion of treatment with protocols other than the recommended three-injection protocol have not been evaluated.

Obtaining both CBC and blood chemistry values allows assessment of liver and kidney function prior to the clinical course of action. The clinician, however, may decide not to perform these tests if cost is deemed an issue and a physical exam reveals the dog to be in overall good health.

Thoracic radiographs and echocardiography allow for assessment of the progression of disease. Most likely, the results of these imaging modalities will not affect the treatment of Class 1–3 dogs.

Melarsomine-Based Treatment

- **AHS-recommended protocol (see Table 2)**
  - Kills 99% of all stages of heartworms (Keister et al., 1992, 1995).
  - Post-treatment respiratory complication rate 6.5%, mortality <0.01% (Nelson et al., 2017; Romito, et al., 2023).
  - Treatment protocol resulted in fewer arterial lesions and no observed thrombi (Kramer et al., 2011).
  - No residual imaging abnormalities referring to HWD (i.e., the radiographic and echocardiographic findings had normalized in all dogs; Romito, et al., 2023).
  - Total length of treatment (including activity restriction) is 16–18 weeks.

- **Abbreviated AHS Protocol (With the Alteration of Melarsomine Administered Immediately After Completion of the Doxycycline Regimen on Day 30) (Carretón et al., 2019)**
  - Six months post-treatment, no antigen or microfilariae were detected in animals.
  - Moderate activity restriction was recommended during the first 30 days and strict activity restriction was recommended for the remainder.
  - Moderate or severe side effects were not noted during the course of treatment by the investigators.
  - All patients were discharged on day 90 with no clinical signs or microfilaremia noted.
  - Total length of treatment (including activity restriction) is 12–14 weeks.

- **Three-Injection Melarsomine Protocol Without Preceding or Concurrent Administration of Doxycycline and Heartworm Preventive**
  - Kills 99% of adult heartworms (Keister et al., 1992, 1995).
  - Post-treatment respiratory complication rate was 20.5% of Class 3 dogs (Case, 1995), with mortality 18.2% in Class 3 dogs (product insert).
  - Total length of treatment (including activity restriction) is 8–10 weeks.

- **Two-Injection Melarsomine Protocol Without Preceding or Concurrent Administration of Doxycycline and Heartworm Preventive**
  - Kills 90% of adult heartworms and 76–81% seroconvert to antigen negative (Keister et al., 1992).
    * Any dog remaining antigen positive will require repeat treatment
  - Post-treatment respiratory complication rate was 27% in Class 2 dogs (Miller et al., 1995) and mortality 4.1% was in Class 1 & 2 dogs (see Immiticide/Diroban product inserts)
  - Total length of treatment (including activity restriction) is 4–6 weeks.

Non-Arsenical ("Slow-Kill") Approaches

There is consensus, based on studies, that all non-arsenical protocols must include doxycycline, a ML, AND activity restriction. Experts would agree that at least 28 days of doxycycline PO BID at 10 mg/kg should be prescribed at the beginning of all of these protocols and should be repeated every 6 months.

The two MLs that have been evaluated are ivermectin and moxidectin (see summary of data below); milbemycin oxime and selamectin have not been evaluated in conjunction with doxycycline.

Please note that animals MUST be activity restricted throughout treatment.

In multiple clinical studies, respiratory complication rates range from 25% to 36% (Ames et al., 2020; Savadelis et al., 2017). In one study in client-owned animals in which activity restriction was not enforced (Ames et al., 2020), coughing during the treatment period was twice (36%) the reported rate of treatment with melarsomine alone and also higher than the rate reported in the AHS recommended treatment protocol described below (see Table 2).

When to Consider (or Not Consider) a Non-Arsenical ("Slow-Kill") Approach for Heartworm Treatment

Indications:

- Dog has previously had an anaphylactic-type reaction to melarsomine
- Dog has a guarded/grave prognosis resulting from some other, non-HW related cause (non-responsive organ disease, terminal neoplasia, etc.)

Contraindications:

- In working dog/performance dog or dog that cannot be activity restricted
- When an ML-resistant infection suspected
- **Use with caution in cases where compliance failure likely led to the HW-positive dog**

Testing Recommendations with Non-Arsenical Protocols

- Test with standard antigen test at 10 months after starting treatment (Savadelis et al., 2017).
  - If positive, retest in 3 months.
  - If NAD, retest with a heat-treated antigen test. If that test is NAD then the animal is considered heartworm free.
  - If the heat-treated antigen test is positive, retest every 3 months until a NAD heat-treated test is obtained.
  - For dogs still testing positive after 18 months consider treating with the two-dose melarsomine protocol.

It is worth noting that 33% of the patients presented at a cardiology referral practice for caval syndrome were undergoing slow-kill heartworm treatment (Vila & Alost, 2023).

Please note that there are a variety of protocols that have been reported in the literature. All studies cannot be reported here, but many articles are freely available at https://pubmed.ncbi.nlm.nih.gov/.

Moxidectin + Doxycycline

For a summary of protocols, please see Jacobson & DiGangi (2021).

Experimental Study (Savadelis et al., 2017)

- Necropsy confirmation of worm death/absence
- Topical moxidectin (2.5 mg/kg monthly) plus doxycycline (10 mg/kg BID for 30 days)
  - Five of 8 dogs had no heartworms at 10 months
  - 96% effective in eliminating adult heartworms (necropsy-based data)
  - Post-treatment respiratory complication rate of 25%
  - Arterial thrombus was scored as a 3.9/4.0 at 10 months, significantly higher in the treated dogs vs nontreated dogs.
  - Radiographic evidence of disease progressed in treated dogs at the same rate as nontreated controls

Clinical Studies (Alberigi et al., 2020; Ames et al., 2020; Ciuca et al., 2023) in client-owned animals, no necropsy confirmation

- Topical moxidectin (2.5 mg/kg twice monthly for 3 months, followed by monthly) plus doxycycline (at least 10 mg/kg BID for 15 days) (Ames et al., 2020)
  - Not activity restricted
  - Post-treatment respiratory complication rate of 36%
  - 95% seroconverted to antigen negative (no heat-treatment), median time 234 days
- Topical moxidectin (2.5 mg/kg monthly for 10 total doses) plus doxycycline (10 mg/kg SID for 30 days) (Ciuca et al., 2023)
  - MF negative by Day 60
  - 8/10 dogs achieved 2 consecutive NAD antigen tests (no heat-treatment) administered 1 month apart; however, 4 of those dogs did have at least 1 positive antigen test with subsequent monthly testing.
  - 9/10 dogs had improvement in thoracic radiograph scores 6 months after initiation of treatment; 1 dog had the same score (score 0 of 3) prior to and 6 months after treatment.
  - 1/10 dogs had improvement in cardiac ultrasound scores 6 months after initiation of treatment; 9 dogs had the same score (7 with scores of 0 of 3, 2 with scores 1 of 3) prior to and 6 months after treatment
- Injectable moxidectin (0.5 mg/kg SQ every 6 months) plus doxycycline (10 mg/kg BID for 30 days) every 6 months until testing NAD with 2 consecutive antigen tests 6 months apart (no heat-treatment) (Alberigi et al., 2020)
  - Of 20 dogs, no antigen was detected in 11 dogs after 6 months, in another 7 by 12 months, another 1 by 18 months, and the last 1 at 27 months.
  - Authors report that respiratory conditions improved.
- Injectable moxidectin (0.17 mg/kg SQ at 0 and 6 months) plus doxycycline (10 mg/kg SID for 30 days) (Ciuca et al., 2023)
  - MF negative by Day 90
  - 9/10 dogs achieved 2 consecutive NAD antigen tests (no heat-treatment) administered 1 month apart; however, 3 of those dogs did have at least 1 positive antigen test with subsequent monthly testing.
  - 6/10 dogs had improvement in thoracic radiograph scores 6 months after initiation of treatment; 4 dogs had the same score (2 with scores of 0 of 3, 2 with scores 1 of 3) prior to and 6 months after treatment.

- 1/10 dogs had improvement in cardiac ultrasound scores 6 months after initiation of treatment; 9 dogs had the same score (6 with scores of 0 of 3, 3 with scores 1 of 3) prior to and 6 months after treatment.

- Oral moxidectin (3 µg/kg monthly for 10 total doses) plus doxycycline (10 mg/kg SID for 30 days) (Ciuca et al., 2023)
  - MF negative by Day 120
  - 6/10 dogs achieved 2 consecutive NAD antigen tests (no heat-treatment) administered 1 month apart; however, 3 of those dogs did have at least 1 positive antigen test with subsequent monthly testing.
  - 7/10 dogs had improvement in thoracic radiograph scores 6 months after initiation of treatment; 3 dogs had the same score (2 with scores 0 of 3, 1 with score 1 of 3) prior to and 6 months after treatment.

Table 3. Summary of Safety-Net (Reach-Back, Retroactive, Clinical Prophylactic), and Adulticidal Activity of Macrocyclic Lactones on *Dirofilaria immitis**

Drug	Age of Heartworms (mos)	Number of Treatments	% Efficacy***	Appearance and/or Motility of Live Heartworms	Reference
Ivermectin (6 µg/kg, per os, monthly)	2	1	100	ND	McCall et al., 1986
	3	13	97.7	abnormal	McCall et al., 1996
	3.5	12	97.8	ND	Bowman et al., 2001
	4	14	97.8	abnormal	McCall et al., 1995
	4	12	95.1	abnormal	McCall et al., 1995
	4.5	12	86.2	ND	Bowman et al., 2001
	5	31	98.7	abnormal	McCall et al., 2001a
	5.5	12	52.2	ND	Bowman et al., 2001
	7	29	94.9	abnormal	McCall et al., 2001a
	8	16	56.3	abnormal	McCall et al., 1998
Milbemycin (500 µg/kg, per os, monthly)	2	1	95.1	ND	Grieve et al., 1991
	2	2	100	ND	Grieve et al., 1991
	3	13	96.7	normal	McCall et al., 1996
	3.5	12	56.5	ND	Bowman et al., 2001
	4	14	49.3	normal	McCall et al., 1995
	4	12	41.4	normal	McCall et al., 1996
	4.5	12	12.7	ND	Bowman et al., 2001
	5.5	12	1.1	ND	Bowman et al., 2001
	6.5	12	15.9	ND	Bowman et al., 2001
	8	16	0	normal	McCall et al., 1998
Selamectin (6 mg/kg topically, monthly)	2	1	100	ND	McTier et al., 2000
	3	12	98.5	ND	McCall et al., 2001c
	adult	18	39.0	abnormal	Dzimianski et al., 2001
Moxidectin (0.5 µg/kg, per os)**	2	1	100	ND	McTier et al., 1992
Moxidectin (0.17 mg/kg SQ, every 6 months)	4	1	85.9	abnormal	McCall et al., 2001d
	4/10	2	97.2	abnormal	McCall et al., 2001d
	6	1	25	abnormal	McCall et al., 2001d
	6/12/18	3	25	abnormal	McCall et al., 2001d

ND = Not done; NA = Not applicable.
* Modified from McCall et al., 2001a
** Please note the currently available FDA-approved formulations of oral moxidectin contain either 12 or 24 µg/kg to be administered monthly per os.
***% efficacy translates to total percentage of worms killed among all dogs evaluated, not % of worm-free dogs in the study

- 2/10 dogs had improvement in cardiac ultrasound scores 6 months after initiation of treatment; 8 dogs had the same score (6 with scores of 0 of 3, 2 with scores 1 of 3) prior to and 6 months after treatment.

Ivermectin + Doxycycline

- Clinical study (Grandi et al., 2010): Client-owned animals, no necropsy confirmation
  - Oral ivermectin (6 µg/kg every 15 days for 6 months; product also contained pyrantel) plus doxycycline (10 mg/kg SID for 30 days)
  - All 11 dogs negative for MF by Day 90
  - 8/11 were antigen NAD by Day 300 (no heat-treatment)
  - Authors reported treatment was well tolerated

Macrocyclic Lactone Only (No Doxycycline Prior to or Concurrent With)

Any methods using continuous monthly administration of prophylactic doses of any ML alone without concurrent doxycycline are NOT RECOMMENDED. While effective in reducing the life span of sexually immature adult and adult heartworms, the data suggest that the older worms are less susceptible, taking longer to die. The adulticidal effect of MLs has been shown to take more than 2 years of continuous administration before adult heartworms are 95% eliminated, and the timing for rigid activity restriction is unknown with this approach (McCall et al., 2001a). Throughout this period, the infection would persist and pathology would continue to progress (Rawlings et al., 2001). Additional important concerns in using MLs as monotherapy of heartworm-positive dogs is the potential for selection of resistant subpopulations of heartworms (Bowman, 2012; Geary et al., 2011) or not eliminating adult worms that might be present due to an initial resistant-isolate infection.

Studies with multiple MLs and their efficacy against worms of different ages (in months) are shown in **Table 3** (McCall et al., 2001a). This table can provide guidance to practitioners on their choice of ML in slow-kill procedures.

Alternative Therapies

Herbal Therapies

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

Compounded Medications

The use of compounded medications in the prevention and treatment of heartworm disease is seldom justified, not recommended, and in most circumstances, violates the AMDUCA. In addition to the legal ramifications there are concerns with stability and potency with compounded doxycycline (Papich et al., 2013) and MLs and toxicity with compounded arsenicals.

Heartworm antigen testing is the most reliable method of confirming the efficacy of adulticidal therapy.

Confirmation of Adulticide Efficacy

Worms that do survive adulticide treatment are invariably the antigen-producing females (Keister et al., 1992). 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 ML 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 and doxycycline was not given during adulticide therapy, or a new infection due to a lapse in administration of preventive.

Heartworm antigen testing is the most reliable method of confirming the efficacy of adulticidal therapy and should be performed 9 months following the last dose of melarsomine. Because adult worms may continue to die for more than a month following adulticide administration, dogs that are still antigenemic at any time less than 9 months post treatment should be allowed more time to clear antigen before retreatment is considered. However, this single test result does not verify that the dog is negative for heartworms, as larval and/or young adult 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 ML and doxycycline were not administered prior to or initiated concurrently with adulticidal therapy. If a heartworm-positive dog is immediately treated with adulticide and a ML is not given until 3 to 4 weeks after the last dose of adulticide, the dog should have a negative antigen test and microfilaria test at the 9-month post-treatment evaluation.

A positive heat-treated antigen result in a dog that has undergone treatment with either melarsomine or "slow-kill" may be a result of the presence of viable worms or dead worms, and further diagnostics and consideration is warranted in making further treatment recommendations.

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 postponed 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. Anesthetic protocols for dogs with varying degrees of severity related to their heartworm infection are summarized by Quandt (2023).

REFERENCES

Alberigi B, Freitas de Souza CdS, Fernandes JI, Merlo A, Labarthe N. Use of slow-release injectable moxidectin for treatment of *Dirofilaria immitis* infection during pregnancy. *Front Vet Sci*. 2020;6:440.

Ames MK, VanVranken P, Evans C, Atkins CE. Non-arsenical heartworm adulticidal therapy using topical moxidectin-imidacloprid and doxycycline: A prospective case series. *Vet Parasitol*. 2020;282:109099.

Aroch I, Rojas A, Slon P, Lavy E, Segev G, Baneth G. Serological cross-reactivity of three commercial in-house immunoassays for detection of *Dirofilaria immitis* antigens with *Spirocerca lupi* in dogs with benign esophageal spirocercosis. *Vet Parasitol*. 2015;211(3-4):303-5

Atkins CE. Comparison of results of three commercial heartworm antigen test kits in dogs with low heartworm burdens. *J Am Vet Med Assoc*. 2003; 222:1221-1223.

Atkins CE, Keene BW, McGuirk SM. Pathophysiologic mechanism of cardiac dysfunction in experimentally induced heartworm caval syndrome in dogs: an echocardiographic study. *Am J Vet Res*. 1988; 49:403-410.

Atkins CE, Murray MJ, Olavessen LJ, Burton KW, Marshall JW, Brooks CC. Heartworm 'lack of effectiveness' claims in the Mississippi Delta: Computerized analysis of owner compliance – 2004–2011. *Vet Parasitol*. 2014;206:106–113.

Atwell RB, Buoro IBJ. Caval syndrome. In Boreman PFL, Atwell RB (eds): *Dirofilariasis*. Boca Raton, FL: CRC Press, 1988, pp 191-203.

Atwell RB, Tarish JH. The effect of oral, low-dose prednisolone on the extent of pulmonary pathology associated with dead *Dirofilaria immitis* in a canine lung model. In *Proceedings of the Heartworm Symposium '95*, Auburn, AL. American Heartworm Society, 1995, pp 103-111.

Badertscher RR, Losonsky JM, Paul AJ, Kneller SK. Two-dimensional echocardiography for diagnosis of dirofilariasis in nine dogs. *J Am Vet Med Assoc*. 1988;193:843-846.

Bandi C, McCall JW, Genchi C, Corona S, Venco L, Sacchi L. Effects of tetracycline on the filarial worms *Brugia pahangi* and *Dirofilaria immitis* and their bacterial endosymbionts *Wolbachia*. *Int J Parasitol*. 1999;29:357-364.

Basile A, Napoli E, Brianti E, Venco L. Right pulmonary artery distensibility index in heartworm infected dogs: Are the different methods leading to same results? *Animals (Basel)*. 2023;13(3):418.

Bazzocchi C, Mortarino M, Grandi G, Kramer LH, Genchi C, Bandi C, Genchi M, Sacchi L, McCall JW. Combined ivermectin and doxycycline treatment has microfilaricidal and adulticidal activity against *Dirofilaria immitis* in experimentally infected dogs. *Int J Parasitol*. 2008;38:1401-1410.

Benedict MQ, Levine RS, Hawley WA, Lounibos LP. Spread of the tiger: global risk of invasion by the mosquito *Aedes albopictus*. *Vector Borne Zoonotic Dis*. 2007;7:76-85.

Bolling BG, Moore CG, Anderson SL, Blair CD, Beaty BJ. Entomological studies along the Colorado Front Range during a period of intense West Nile virus activity. *J Am Mosq Control Assoc*. 2007;23(1):37-46.

Bouchery T, Lefoulon E, Karadjian G, Nieguitsila A, Martin C. The symbiotic role of *Wolbachia* in Onchocercidae and its impact on filariasis. *Clin Microbiol Infect*. 2013;19:131-140.

Boudreaux M, Dillon AR, Ravis WR, Sartin EA, Spano JS. Effects of treatment with aspirin or aspirin/dipyridamole combination in heartworm-negative, heartworm infected, and embolized heartworm–infected dogs. *Am J Vet Res*. 1991; 52(12):1992-1999.

Bourguinat C, Lee ACY, Lizundia R, Blagburn BL, Liotta JL, Kraus MS, Keller K, Epe C, Letourneau L, Kleinman CL, Paterson T, Gomez EC, Montoya-Alonso JA, Smith H, Bhan A, Peregrine AS, Carmichael J, Drake J, Schenker R, Kaminsky R, Bowman DD, Geary TG, Prichard RK. Macrocyclic lactone resistance in *Dirofilaria immitis*: failure of heartworm preventives and investigation of genetic markers for resistance. *Vet Parasitol*. 2015;210(3-4):167-178.

Bowman DD. Heartworms, macrocyclic lactones, and the specter of resistance to prevention in the United States. *Parasit Vectors*. 2012;5:138.

Bowman DD, Atkins CE. Heartworm biology, treatment, and control. *Vet Clin North Am Small Anim Pract*. 2009;39:1127-1158.

Bowman DD, Little SE, Lorentzen L, Shields J, Sullivan MP, Carlin EP. Prevalence and geographic distribution of *Dirofilaria immitis*, *Borrelia burgdorferi*, *Ehrlichia canis*, and *Anaplasma phagocytophilum* in dogs in the United States: Results of a national clinic-based serologic survey. *Vet Parasitol*. 2009;160:138-148.

Bowman DD, Neumann N R, Rawlings C, Stansfield DG, Legg W. Effects of avermectins on microfilariae in dogs with existing and developing heartworm infections. In *Recent Advances in Heartworm Disease '01*. American Heartworm Society, 2001, pp. 173-178.

Boysen SR, Lisciandro GR. The use of ultrasound for dogs and cats in the emergency room: AFAST and TFAST. *Vet Clin North Am Small Anim Pract*. 2013;43(4):773-97.

Budde JA, McCluskey DM. Doxycycline. In *Plumb's Veterinary Drug Handbook*, 10th ed. Hoboken, NJ: Wiley-Blackwell, 2023, pp 433-438.

Calvert CA. Treatment of heartworm disease with associated severe pulmonary arterial disease. In *Proceedings of the Heartworm Symposium '86*, New Orleans. American Heartworm Society, 1986, pp 125-129.

Calvert CA, Rawlings CA. Canine heartworm disease. In Fox PR (ed): *Canine and Feline Cardiology*. New York: Churchill Livingstone, 1988, pp 519-549.

Campbell WC (ed). *Ivermectin and Abamectin*. New York: Springer-Verlag; 1989, pp 248-250.

Carithers DS. Examining the role of macrolides and host immunity in combatting filarial parasites. *Parasit Vectors*. 2017;10:182.

Carretón W, Falcón-Cordón Y, Falcón-Cordón S, Morchón R, Matos JI, Montoya-Alonso JA. Variation of the adulticide protocol for the treatment of canine heartworm infection: can it be shorter? *Vet Parasitol*. 2019;271:54-56.

Case JL, Tanner PA, Keister DM, Meo NJ. A clinical field trial of melarsomine dihydrochloride (RM340) in dogs with severe (class 3) heartworm disease. In *Proceedings of the Heartworm Symposium '95*, Auburn, AL. American Heartworm Society, 1995, pp 243-250.

Christensen BM, Hollander AL. Effect of temperature on vector parasite relationships of *Aedes trivittatus* and *Dirofilaria immitis*. *Proceedings of the Helminthological Society of Washington*. 1978;45:115-119.

Christensen BM, Rowley WA. Observations on the laboratory biology and maintenance of *Aedes trivittatus*. *Mosquito News*.1978;38:9-14.

Ciuca L, Vismarra A, Constanza D, Di Loria A, Meomartino L, Ciaramella P, Cringoli G, Genchi M, Rinaldi L, Kramer L. Efficacy of oral, topical, and extended-release injectable formulations of moxidectin combined with doxycycline in *Dirofilaria immitis* naturally infected dogs. *Parasit Vectors*. 2023;16(1):54.

Couper LI, Mordecai EA. Ecological drivers of dog heartworm transmission in California. *Parasit Vectors*. 2022;15(1):388.

Courtney CH. Predicting heartworm burdens with a heartworm antigen test kit. *JAAHA*. 1987;23:387-390.

Courtney CH, Cornell JA. Evaluation of heartworm immunodiagnostic tests. *J Am Vet Med Assoc*. 1990;197:724-729.

Courtney CH, Zeng Q-Y. Comparison of heartworm antigen test kit performance in dogs having low heartworm burdens. *Vet Parasitol*. 2001;96:317-322.

Darsie R Jr, Ward R. *Identification and Geographical Distribution of the Mosquitoes of North America, North of Mexico*. University Press of Florida, Gainesville, FL, 2005.

Dey S, Kurade NP, Khurana KL, Dan A. Clinicobiochemical changes in ivermectin toxicity in Doberman pinscher pups. *J Parasit Dis*. 2017;41(2):580–3.

DiGangi BA, Dworkin C, Stull JW, O'Quin J, Elser M, Marsh AE, Groshong L, Wolfson W, Duhon B, Broaddus K, Gingrich EN, Swiniarski E, Berliner EA. Impact of heat treatment on *Dirofilaria immitis* antigen detection in shelter dogs. *Parasit Vectors*. 2017;10(Suppl 2):483.

Dillon AR, Brawner WR, Hanrahan L. Influence of number of parasites and exercise on the severity of heartworm disease in dogs. In *Proceedings of the Heartworm Symposium '95*, Auburn, AL. American Heartworm Society, 1995a, p 113.

Dillon AR, Warner AE, Molina RM. Pulmonary parenchymal changes in dogs and cats after experimental transplantation of dead *Dirofilaria immitis. In Sol MD, Knight DH (eds): Proceedings of the Heartworm Symposium*, Auburn, AL. American Heartworm Society, 1995b, pp 97-101.

Dorman DC. Diethyltoluamide (DEET) insect repellent toxicosis. *Vet Clin North Am Small Anim Pract*. 1990;20(2):387-91.

Duncan K, Barrett AW, Little SE, Sundstrom KD, Guerino F. Fluralaner (Bravecto®) treatment kills *Aedes aegypti* after feeding on *Dirofilaria immitis* infected dogs. *Parasit Vectors*. 2023;16:208.

Dzimianski MT, McCall JW, Mansour AM. The effect of prednisone on the efficacy of melarsomine dihydrochloride against adult *Dirofilaria immitis* in experimentally infected beagles. In *State of the Heartworm '10 Symposium*, Memphis, TN. American Heartworm Society, 2010.

Dzimianski MT, McCall JW, McTier TL, Raynaud JP. Efficacy of RM 340 compared with thiacetarsamide judged by objective criteria. 1. Controlled laboratory tests in canine models. In *Proceedings of the AAVP 35th Annual Meeting*, San Antonio, TX, 1990, p 51.

Dzimianski MT, McCall JW., Steffens, Supakorndej N, Mansour AE, Ard MB, McCall SD, Hack R. The safety of selamectin in heartworm infected dogs and its effect on adult worms and microfilariae. In *Recent Advances in Heartworm Disease 01*. San Antonio, Texas, American Heartworm Society, 2001, pp. 135-140.

Dzimianski MT, McTier TL, McCall JW, Raynaud JP. Assessment of filaricidal activity of a new filaricide (RM 340) against immature and adult heartworms using experimental canine models. In *Proceedings of the Heartworm Symposium '89*, Washington, DC. American Heartworm Society, 1989, pp 147-153.

Ernst J, Slocombe JOD. The effect of low temperature on developing *Dirofilaria immitis* larvae in *Aedes triseriatus*. In *Proceedings of the Heartworm Symposium '83*, Orlando, FL. American Heartworm Society, 1983, pp 1-4.

Evans CC, Bradner JL, Savadelis MD, Nelson CT, Moorhead AR. Glacial acetic acid as an alternative reagent for the modified Knott test. *Vet Parasitol*. 2019;276:108975.

Evans CC, Normille D, Gamble S, Guerino F, Dzimianski MT, Moorhead AR. Treatment of dogs with Bravecto® (fluralaner) reduces mosquito survival and fecundity. *Parasit Vectors*. 2023;16:147.

Farajollahi A, Crans WJ, Bryant P, Wolf B, Burkhalter KL, Godsey MS, Aspen SE, Nasci RS. Detection of West Nile Viral RNA from an overwintering pool of *Culex pipens pipiens* (Diptera: Culicidae) in New Jersey, 2003. *J Med Ent.* 2005;42:490-494.

Fortin JF, Slocombe JOD. Temperature requirements for the development of *Dirofilaria immitis* in *Aedes triseriatus* and *Ae. vexans. Mosquito News*. 1981;41:625-633.

Fukami N, Hagio M, Okano S, Watanabe S. Influence of exercise on recovery of dogs following heartworm adulticide treatment with melarsomine. In *Recent Advances in Heartworm Disease: Symposium '98*, Tampa, FL. American Heartworm Society, 1998, pp 225-227.

Geary TG, Bourguinat C, Prichard RK. Evidence for macrocyclic lactone anthelmintic resistance in *Dirofilaria immitis*. *Topics Companion Anim Med*. 2011;26:186-192.

Genchi M, Ciuca L, Vismarra A, Ciccone E, Cringoli G, Kramer L, Rinaldi L. Evaluation of alternative reagents on the performance of the modified Knott's test. *Vet Parasitol*. 2021;298:109555.

Georgi JR, Georgi ME. Heartworms and other filarids. In *Canine Clinical Parasitology*. Philadelphia, PA: Lea & Febiger, 1992, pp 192-198.

Geurden T, Chapin S, McCall JW, Mansour A, Hahabir SP, Kryda K, McTier T. Insecticidal activity of Simparica and Simparica Trio against *Aedes aegypti* in dogs. *Parasit Vectors*. 2023;16:95.

Gjullin CM, Yates WW, Stage HH. Studies on *Aedes vexans* (Meig.) and *Aedes sticticus* (Meig.) floodwater mosquitoes in the lower Columbia River Valley. *Ann Entomol Soc Am*. 1950;43:262-275.

Grandi G, Quintavalla C, Mavropoulou A, Genchi M, Gnudi G, Bertoni G, Kramer L. A combination of doxycycline and ivermectin is adulticidal in dogs with naturally acquired heartworm disease (*Dirofilaria immitis*). *Vet Parasitol*. 2010;169:347-351.

Grieve, RB, Grank GR, Stevart VA, et al. Chemoprophylactic effects of milbemycin oxime against larvae of *Dirofilaria immitis* during prepatent development. *Am J Vet Res*. 1991;52(12):2040-2042.

Grieve RB, Knight DH. Anti-*Dirofilaria immitis* antibody levels before and after anthelmintic treatment of experimentally infected dogs. *J Parasitol*. 1985;71:56-61.

Gruntmeir JM, Long MT, Blagburn BL, Walden HS. Canine heartworm and heat treatment: An evaluation using a well based enzyme-linked immunosorbent assay (ELISA) and canine sera with confirmed heartworm infection status. *Vet Parasitol*. 2020;283:109169.

Gwaltney-Brant, S. Insecticides and Molluscicides. In Plumlee KH (ed.): *Clinical Veterinary Toxicology*. St. Louis: Mosby, 2004; pp 177-192.

Hanson SM, Craig GB. *Aedes albopictus* (Diptera: Culcidae) eggs: Field survivorship during northern Indiana winters. *J Med Ent*. 1995;32(5);599-604.

Hawley WA, Pumpuni CB, Brady RH, Craig Jr. GB. Overwintering survival of *Aedes albopictus* (Diptera: Culicidae) eggs in Indiana. *J Med Ent*. 1989;26(2):122-129.

Hinman EH, Hurlbut HS. A study of winter activities and hibernation of *Anopheles quadrimaculatus* in the Tennessee Valley. *Am J Trop Med Hyg*. 1940;20:431-446.

Hirano Y, Kitagawa H, Sasaki Y. Relationship between pulmonary arterial pressure and pulmonary thromboembolism associated with dead worms in canine heartworm disease. *J Vet Med Sci*. 1992;54:897-904.

Hoerauf A, Mand S, Fischer K, Kruppa T, Marfo-Debrekyei Y, Debrah AY, Pfarr KM, Adjei O, Büttner DW. Doxycycline as a novel strategy against bancroftian filariasis-depletion of *Wolbachia* endosymbionts from *Wuchereria bancrofti* and stop of microfilaria production. *Med Microbiol Immunol*. 2003;192:211-216.

Hoffman J, Miller JR. Reassessment of the role and utility of wind in suppression of mosquito (Diptera: Culicidae) host finding: Stimulus dilution supported over flight limitation. *J Med Entomol*. 2003;40(5):607-614.

Holderman C, Abruzzo NO, Abdelsamad NA, Kaufman PE, DiGennaro PM. Collection and DNA detection of *Dirofilaria immitis* (Rhabditida Onchocercidae), using a novel primer set, in wild-caught mosquitoes from Gainesville, FL. *J Med Ent*omol. 2021;58(3):1429-1432.

Hoskins JD, Hribernik TN, Kearney MT. Complications following thiacetarsamide sodium therapy in Louisiana dogs with naturally-occurring heartworm disease. *Cornell Vet*. 1985;75:531-539.

Hudson JE. Cold hardiness of some adult mosquitoes in central Alberta. *Can J Zool*. 1978;56(8):1697-1709

Ishihara K, Kitagawa H, Ojima M, Yagata Y, Suganuma Y. Clinicopathological studies on canine dirofilarial hemoglobinuria. *Jap J Vet Sci*. 1978;40:525-537.

Ishihara K, Kitagawa H, Sasaki Y. Efficacy of heartworm removal in dogs with dirofilarial hemoglobinuria using flexible alligator forceps. *Jap J Vet Sci*. 1988;50:739-745.

Jackson RF. The venae cavae syndrome. In Otto G, Jackson RF, Jackson WF (eds): *Proceedings of the Heartworm Symposium 1974*, Auburn, AL. American Heartworm Society, 1974, pp 48-50.

Jackson RF, Seymour WG, Growney PJ, Otto GF. Surgical treatment of the caval syndrome of canine heartworm disease. *J Am Vet Med Assoc*. 1977;171:1065-1069.

Jacobson LS, DiGangi BA. An accessible alternative to melarsomine: "Moxi-Doxy" for treatment of adult heartworm infection in dogs. *Front Vet Sci*. 2021;8:702018.

Johnson C, Padgett K. Heartworm prevention: clients can't comply if they don't know they should. *Vet Pract News*. 2019;Sept.

Jones SL. Canine caval syndrome series, Part 3: Management of caval syndrome. *Today's Vet Pract*. 2016;May/June.

Kartman L. Factors influencing infection of the mosquito with *Dirofilaria immitis* (Leidy, 1856). *Exp Parasitol*. 1953;2:27-78.

Keister DM, Dzimianski MT, McTier TL, et al. Dose selection and confirmation of RM 340, a new filaricide for the treatment of dogs with immature and mature *Dirofilaria immitis*. In *Proceedings of the Heartworm Symposium '92*, Austin, TX. American Heartworm Society, 1992, pp 225-229.

Keister DM, Tanner PA, Meo NJ. Immiticide: Review of discovery, development and Utility. In *Proceedings of the Heartworm Symposium '95*. Auburn, Alabama, American Heartworm Society, 1995, pp. 201-219.

Khan SU, Ogden NH, Fazil AA, Gachon PH, Dueymes GU, Greer AL, Ng V. Current and Projected Distributions of *Aedes aegypti* and *Ae. albopictus* in Canada and the U.S. *Environ Health Perspect*. 2020;128(5):57007.

Kim J, Jeong J, Park K, Shin K, Jang IS, Yoon H. Evaluation of improved transvenous heartworm extraction brush in dogs with caval syndrome. *J Vet Sci*. 2023;24(4):e46.

Kitagawa H, Sasaki Y, Ishihara K. Clinical studies on canine dirofilarial hemoglobinuria: relationship between the presence of heartworm mass at the tricuspid valve orifice and plasma hemoglobin concentration. *Jap J Vet Sci*. 1986;48:99-103.

Knight DH. Guidelines for diagnosis and management of heartworm (*Dirofilaria immitis*) infection. In Bonagura JD (ed): *Kirk's Current Veterinary Therapy XII, Small Animal Practice*. Philadelphia, PA: WB Saunders Co, 1995, pp 879-887.

Knight DH, Lok JB. Seasonality of heartworm infection and implications for preventive. *Clin Tech Small Anim*. 1998;13:77-82.

Knott J. A method for making microfilarial surveys on day blood. *Trans R Soc Trop Med Hyg*. 1939;33:191-196.

Kotani T, Powers KG. Developmental stages of *Dirofilaria immitis* in the dog. *Am J Vet Res*. 1982;43:2199-2206.

Kozek WJ. What is new in the *Wolbachia*/*Dirofilaria* interaction. *Vet Parasitol*. 2005;133(2-3):127-132.

Kozek WJ, Vazquez AE, Gonzalez C Jr, Inguina J, Sanchez E, de Jesus F, Cardona, CJ Jr, Gomez C, Senirez R, Diaz-Umpierre. Prevalence of canine filariae in Puerto Rico and the Caribbean. In *Proceedings of the Heartworm Symposium '95*, Auburn, AL. American Heartworm Society, 1995.

Kramer L, Grandi G, Passeri B, Gianelli P, Genchi M, Dzimianski MT, Supakorndej P, Mansour AM, Supakorndej, McCall SD, McCall JM. Evaluation of lung pathology in *Dirofilaria immitis* – Experimentally infected dogs treated with doxycycline or a combination of doxycycline and ivermectin before administration of melarsomine dihydrochloride. *Vet Parasitol*. 2011;176:357-360.

Kramer L, Simón F, Tamarozzi F, Genchi M, Bazzocchi C. Is *Wolbachia* complicating the pathological effects of *Dirofilaria immitis* infections? *Vet Parasitol*. 2005a;133(2-3):133-136.

Kramer LH, Tamarozzi F, Morchón R, López-Belmonte J, Marcos-Atxutegi C, Martín-Pacho R, Simón F. Immune response to and tissue localization of the *Wolbachia* surface protein (WSP) in dogs with natural heartworm (*Dirofilaria immitis*) infection. *Vet Immunol Immunopathol*. 2005b;106:303-308.

Kume S, Itagaki S. On the life-cycle of *Dirofilaria immitis* in the dog as the final host. *Br Vet J*. 1955;111:16-24.

Ledesma N, Harrington L. Fine-scale temperature fluctuation and modulation of *Dirofilaria immitis* larval development in *Aedes aegypti*. *Vet Parasitol*. 2015;209(1–2):93–100.

Lee ACY, Bowman DD, Lucio-Forster A, Beall MJ, Liotta JL, Dillon R. Evaluation of a new in-clinic method for the detection of canine heartworm antigen. *Vet Parasitol*. 2011;177:387-391.

Lichtenfels JR, Pilitt PA, Kotani T, Powers KG. Morphogenesis of developmental stages of *Dirofilaria immitis* (Nematoda) in the dog. *P Helm Soc Wash*. 1985;52:98-113.

Liebenberg J, Fourie J, Lebon W, Larsen D, Halos L, Beugnet F. Assessment of the insecticidal activity of afoxolaner against *Aedes aegypti* in dogs treated with NexGard®. *Parasite*. 2017;24:39.

Lisciandro GR, Lisciandro SC. Global FAST for patient monitoring and staging in dogs and cats. *Vet Clin North Am Small Anim Pract*. 2021a;51(6):1315-1333.

Lisciandro GR, Lisciandro SC. Lung ultrasound fundamentals, "wet versus dry" lung, signs of consolidation in dogs and cats. *Vet Clin North Am Small Anim Pract*. 2021b;51(6):1125-1140.

Little S, Saleh M, Wohltjen M, Nagamori Y. Prime detection of *Dirofilaria immitis*: understanding the influence of blocked antigen on heartworm test performance. *Parasit Vectors*. 2018;11(1):186.

Long SA, Rhinehart J, Shrake J, Marsh AE. Feasibility and comparative analysis of *Dirofilaria immitis* microfilaria freezing and fixation for student instruction and assessment of clinical parasitology skills. *BMC Vet Res*. 2020;16(1):31.

Löwenberg Neto P, Navarro-Silva MA. Development, longevity, gonotrophic cycle and oviposition of *Aedes albopictus* Skuse (Diptera: Culicidae) under cyclic temperatures. *Neotrop Entomol*. 2004;33:29-33.

Ludlam KW, Jachowski LA Jr, Otto GF. Potential vectors of *Dirofilaria immitis*. *J Am Vet Med Assoc*. 1970;157:1354-1359.

Matos JI, Caro-Vadillo A, Falcón-Cordón Y, García-Rodríguez SN, Costa-Rodríguez N, Carretón E, Montoya-Alonso JA. Echocardiographic assessment of the pulmonary vein to pulmonary artery ratio in canine heartworm disease. *Animals (Basel)*. 2023;13(4):703.

McCall JW. A parallel between experimentally induced canine and feline heartworm disease. In *Proceedings of XVII World Small Animal Veterinary Association World Congress*. Rome, 1992, pp 255-261.

McCall JW. The safety-net story about macrocyclic lactone heartworm preventives: A review, an update, and recommendations. *Vet Parasitol*. 2005;133:197-206.

McCall JW, Arther R, Davis W, Settje T. Safety and efficacy of 10% imidacloprid + 2.5% moxidectin for the treatment of *Dirofilaria immitis* circulating microfilariae in experimentally infected dogs. *Vet Parasitol*. 2014b;206;5-13.

McCall, JW, DiCosty, U, Mansour A, Fricks C, McCall S, Dzimianski MT, Carson B. Inability of *Dirofilaria immitis* infective larvae from mosquitoes fed on blood from microfilaremic dogs during low-dose and short-treatment regimens of doxycycline and ivermectin to complete normal development in heartworm naïve dogs. *Parsit Vectors*. 2023b;16:199.

McCall JW, Dzimianski MT, Plue RE, Seward RL, Blair LS. Ivermectin in heartworm prophylaxis: Studies with experimentally induced and naturally acquired infections. In *Proceedings of the Heartworm Symposium '86*. New Orleans, Louisiana, 1986. American Heartworm Society, pp. 9-13.

McCall JW, Genchi C, Kramer L, Guerrero J, Dzimianski MT, Supakorndej P, Mansour AM, McCall SD, Supakorndej N, Grandi G, Carson B. Heartworm and *Wolbachia*: therapeutic implications. *Vet Parasitol*. 2008a;158:204-214.

McCall JW, Genchi C, Kramer LH, Guerrero J, Venco L. Heartworm disease in animals and humans. In *Rollinson D, Hay SI (eds): Advances in Parasitology*. New York: Academic Press, 2008b, pp 193-285.

McCall JW, Guerrero J, Roberts RE, Supakorndej N, Mansour AE, Dzimianski MT, McCall SD. Further evidence of clinical prophylactic, retroactive (reach-back) and adulticidal activity of monthly administrations of ivermectin (Heartgard Plus) in dogs experimentally infected with heartworms. In *Recent Advances in Heartworm Disease Symposium '01*. American Heartworm Society, 2001a, pp 198-200.

McCall JW, Hack R, McCall SD, Mansour AE, Supakorndej N, Supakorndej P, Steffens WL. Evaluation of repeated monthly dosing of selamectin against *Dirofilaria immitis* beginning three months after experimental inoculation of heartworm larvae in dogs. In *In Recent Advances in Heartworm Disease '01*. San Antonio, Texas, American Heartworm Society, 2001c, pp. 141-148.

McCall JW, Hodgkins E, Varloud, Mansour A, DiCosty U. Blocking the transmission of heartworm (*Dirofilaria immitis*) to mosquitoes (*Aedes aegypti*) by weekly exposure for one month to microfilaremic dogs treated once topically with dinotefuran-permethrin-pyriproxyfen. *Parasit Vectors*. 2017a;10(Suppl 2):511.

McCall JW, Kramer L, Genchi C, Guerrero J, Dzimianski MT, Supakorndej P, Mansour A, McCall SD, Supakorndej N, Grandi G, Carson B. Effects of doxycycline on early infections of *Dirofilaria immitis* in dogs. *Vet Parasitol*. 2011;176:361-367.

McCall JW, Kramer L, Genchi C, Guerrero J, Dzimianski MT, Mansour A, McCall SD, Carson B. Effects of doxycycline on heartworm embryogenesis, transmission, circulating microfilaria, and adult worms in microfilaremic dogs. *Vet Parasitol*. 2014a; 206(1-2):5-13.

McCall JW, Mansour A, DiCosty U, Fricks C, McCall S, Dzimianski MT, Carson B. Long-term evaluation of viability of microfilariae and intravenously transplanted adult *Dirofilaria immitis* in microfilaremic dogs treated with low-dose, short-and long-treatment regimens of doxycycline and ivermectin. *Parasit Vectors*. 2023a;16:190.

McCall, JW, McTier TL, Ryan WG, Gross SJ, Soll MD. Evaluation of ivermectin and milbemycin oxime efficacy against *Dirofilaria immitis* infections of three and four months duration. *Am J Vet Res*, 1996, pp. 1189-1192.

McCall JW, McTier TL, Supakorndej N, Ricketts R. Clinical prophylactic activity of macrolides on young adult heartworms. In *Proceedings of the Heartworm Symposium '95*. Auburn, Alabama, American Heartworm Society, 1995, pp. 187-195.

McCall JW, Ryan WG, Roberts RE, Dzimianski MT. Heartworm adulticidal activity of prophylactic doses of ivermectin (6 mcg/kg) plus pyrantel administered monthly to dogs. In *Recent Advances in Heartworm Disease '98*. Tampa, Florida, American Heartworm Society, 1998, pp.209-215.

McCall JW, Supakorndej N, Donoghue AR, Turnbull RK, Radecki SV. Evaluation of the performance of canine heartworm antigen test kits licensed for use by veterinarians and canine heartworm test kits conducted by diagnostic laboratories. In *Recent Advances in Heartworm Disease: Symposium '01*. American Heartworm Society, 2001b, pp 97-104.

McCall JW, Supakorndej P, Dzimianski MT, Supakorndej N, Mansour AE, Jun JJ, McCall SD, Wang GT, Sinha A, Rulli RD. Evaluation of retroactive and adulticidal activity of moxidectin canine SR (Sustained Release) injectable formulation against *Dirofilaria immitis* in Beagles. In *Recent Advances in Heartworm Disease 01*. San Antonio, Texas, American Heartworm Society, 2001d, pp. 165-172.

McCall JW, Varloud M, Hodgkins E, Mansour A, DiCosty U, McCall S, Carmichael J, Carson B, Carter J. Shifting the paradigm in *Dirofilaria immitis* prevention: blocking transmission from mosquitoes to dogs using repellents/insecticides and macrocyclic lactone prevention as part of a multimodal approach. *Parasit Vectors*. 2017b;10(Suppl 2):525.

McGreevy PB, Theis JH, Lavoipierre MM, Clark J. Studies on filariasis. III. *Dirofilaria immitis*: emergence of infective larvae from the mouthparts of *Aedes aegypti*. *J Helminthol*. 1974;48:221-228.

McKay T, Bianco T, Rhodes L, Barnett S. Prevalence of *Dirofilaria immitis* (Nematoda: Filarioidea) in mosquitoes from northeast Arkansas, the United States. *J Med Ent*omol. 2013;50:871-878.

McTier TL, Holzmer S, Kryda K, Mahabir S, McCall JW, Trombley J, Maeder SJ. Comparative preventive efficacy of ProHeart® 12, Heartgard® Plus and Interceptor® Plus against a macrocyclic lactone-resistant strain (JYD-34) of heartworm (*Dirofilaria immitis*) in dogs. *Parasit Vectors*. 2021;14(1):226.

McTier TL, McCall JW, Dzimianski MT, Aguilar R, Wood I. Prevention of experimental heartworm infection in dogs with single, oral doses of moxidectin. In *Proceedings of the Heartworm Symposium '92*. Austin, Texas, American Heartworm Society, 1992, pp. 165-168.

McTier TL, McCall JW, Dzimianski MT, Raynaud JP, Strickland JE. Use of melarsomine dihydrochloride (RM 340) for adulticidal treatment of dogs with naturally acquired infections of *Dirofilaria immitis* and for clinical prophylaxis during reexposure for 1 year. *Vet Parasitol*. 1994;55:221-233.

McTier TL, Shanks DJ, Watson P, McCall JW, Genchi C, Six RH, Thomas CA, Dickin SK, Pengo G, Rowan TJ, Jernigan AD. Prevention of experimentally induced heartworm *Dirofilaria immitis* infections in dogs and cats with a single topical application of selamectin. *Vet Parasitol*. 2000;91:259-268.

McTier TL, Six RH, Pullins A, Chapin S, Kryda K, Mahabir SP, Woods DJ, Maeder SJ. Preventive efficacy of oral moxidectin at various doses and dosage regimens against macrocyclic lactone-resistant heartworm (*Dirofilaria immitis*) strains in dogs. *Parasit Vectors*. 2019;12(1):444.

Mealey KL. Canine ABCB1 and macrocyclic lactones: Heartworm prevention and pharmacogenetics. *Vet Parasitol*. 2008;158:215-222.

Metzger ME, Wekesa JW, Kluh S, Fujioka KK, Saviskas R, Arugay A, McConnell N, Nguyen K, Krueger L, Hacker GM, Hu R, Kramer VL. Detection and Establishment of *Aedes notoscritpus* (Diptera: Culicidae) Mosquitoes in Southern California, United States. *J Med Ento*mol. 2022;59(1):67–77.

Miller MW, Keister DM, Tanner PA, Meo NJ. Clinical efficacy of melarsomine dihydrochloride (RM 340) and thiacetarsamide in dogs with moderate (Class 2) heartworm disease. In *Proceedings of the Heartworm Symposium '95*. Auburn, Alabama, 1995. American Heartworm Society, pp. 233-241.

Miglianico M, Eldering M, Slater H, Ferguson N, Ambrose P, Lees RS, Koolen KMJ, Pruzinova K, Jancarova M, Volf P, Koenraadt CJM, Duerr H-P, Trevitt G, Yang B, Chatterjee AK, Wisler J, Sturm A, Bousema T, Sauerwein RW, Schultz PG, Tremblay MS, Dechering KJ. Repurposing isoxazoline veterinary drugs for control of vector-borne human diseases. *PNAS*. 2018;115:E6921-E6926.

Moise NS. Echocardiography. In Fox PR (ed): *Canine and Feline Cardiology*. New York: Churchhill Livingstone, 1988, pp 113-156.

Moorhead AR, Evans CC, Kaplan RM. A diagnostic algorithm for evaluating cases of potential macrocyclic lactone–resistant heartworm. *Parasit Vectors*. 2017;10(Suppl 2):479.

Moorhead AR, Evans CC, Sakamoto K, Dzimianski MT, Mansour A, DiCosty U, Fricks C, McCall S, Carson B, Nelson CT, McCall JW. Effects of doxycycline dose rate and pre-adulticide wait period on heartworm-associated pathology and adult worm mass. *Parasit Vectors*. 2023;16(1):251.

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

Moreno Y, Nabhan JF, Solomon J, Mackenzie CD, Geary TG. Ivermectin disrupts the function of the excretory-secretory apparatus in microfilariae of *Brugia malayi*. *Proc Natl Acad Sci USA*. 2010;107:20120-20125.

Nelson CT. Heartworm and related nematodes. In Sykes JE (ed): *Greene's Infectious Diseases of the Dog and Cat*, 5th ed. Elsevier, 2023, pp 1399-1417.

Nelson CT. Evaluation of temperature variation in microclimes in multiple U.S.A. locales and its effect on accuracy of models for prediction of mosquito survival and heartworm transmission. Presented at the American Heartworm Society Triennial Symposium, 2016.

Nelson CT, Myrick ES, Nelson TA. Clinical benefits of incorporating doxycycline into a canine heartworm treatment protocol. *Parasit Vectors*. 2017;10(Suppl 2):515.

Newton WL. Longevity of an experimental infection with *Dirofilaria immitis* in a dog. *J Parasitol.* 1968;54(1):187-8.

Orihel TC. Morphology of the larval stages of *Dirofilaria immitis* in the dog. *J Parasitol.* 1961;47:251-262.

Papich MG, Davidson GS, Fortier LA. Doxycycline concentration over time after storage in a compounded veterinary preparation. *J Am Vet Med Assoc*. 2013;242(12):1674-1678.

Pariaut R, Jung SW, Vila J, Newhard DK. Resolution of caval syndrome during initial hemodynamic stabilization in dogs with heartworm disease. *J Vet Emerg Crit Care* (San Antonio). 2020;30(3):295-301.

Patel A, Khande H, Periasamy H, Mokale S. Immunomodulatory effect of doxycycline ameliorates systemic and pulmonary inflammation in a murine polymicrobial sepsis model. *Inflammation*. 2020;43(3):1035-1043.

Paul AJ, Todd Jr. KS, Sundberg JP, DiPietro JA, McCall JW. Efficacy of ivermectin against *Dirofilaria immitis* larvae in dogs 30 and 45 days after induced infection. *Am J Vet Res*. 1986;47:883-884.

Peterson AT, Campbell LP. Global potential distribution of the mosquito *Aedes notoscriptus*, a new alien species in the United States. *J Vector Ecol*. 2015 Jun;40(1):191-4.

Peterson KM, Chappell DE, Lewis B, Staton A, Dement E, Prater PE, Blanton RA. Heartworm-positive dogs recover without complications from surgical sterilization using cardiovascular sparing anesthesia protocol. *Vet Parasitol*. 2014; 206:83-85.

Pratt HD, Moore CD. *Mosquitoes of Public Health Importance and Their Control*. United States Government Printing Office, Washington, DC, 1960.

Pulaski CN, Moorhead AR, Evans CC, Kaplan RM. Updating the diagnostic algorithm for evaluating cases of suspected macrocyclic lactone-resistant heartworm infection. Presented at the American Heartworm Society Triennial Symposium, 2019.

Pulliam JD, Seward RL, Henry RT, Steinberg SA. Investigating ivermectin toxicity in Collies. *Vet Med*. 1985;80:33-40.

Quandt J. Anesthesia for the dog with heartworm disease: a brief, practical review. *Parasit Vectors*. 2023;16(1):151.

Rawlings CA. Acute response of pulmonary blood flow and right ventricular function to *Dirofilaria immitis* adults and microfilaria. *Am J Vet Res*. 1980;41:244-249.

Rawlings CA. *Heartworm Disease in Dogs and Cats*. Philadelphia: Saunders, 1986.

Rawlings CA, Bowman DD, Howerth EW, Stansfield DG, Legg W, Luempert LG. Response of dogs treated with ivermectin or milbemycin starting at various intervals after *Dirofilaria immitis* infection. *Vet Therap Res Appl Vet Med*. 2001;2:193-207.

Rawlings CA, Losonsky JM, Lewis RE, McCall JW. Development and resolution of radiographic lesions in canine heartworm disease. *J Am Vet Med Assoc*. 1981;178(11):1172–7.

Rawlings CA, Raynaud JP, Lewis RE, Duncan JR. Pulmonary thromboembolism and hypertension after thiacetarsamide vs melarsomine dihydrochloride treatment of *Dirofilaria immitis* infection in dogs. *Am J Vet Res*. 1993b;54:920-925.

Rawlings CA, Tonelli Q, Lewis RE, Duncan JR. Semiquantitative test for *Dirofilaria immitis* as a predictor of thromboembolic complications associated with heartworm treatment in dogs. *Am J Vet Res*. 1993a;54:914-919.

Reinero C, Visser LC, Kellihan HB, Masseau I, Rozanski E, Clercx C, Williams K, Abbott J, Borgarelli M, Scansen BA. ACVIM consensus statement guidelines for the diagnosis, classification, treatment, and monitoring of pulmonary hypertension in dogs. *J Vet Intern Med*. 2020;34(2):549-573.

Romano AE, Saunders AB, Gordon SG, Wesselowski S. Intracardiac heartworms in dogs: Clinical and echocardiographic characteristics in 72 cases (2010-2019). *J Vet Intern Med*. 2021;35(1):88-97.

Romi R, Severini F, Toma L. Cold acclimation and overwintering of female *Aedes albopictus* in Roma. *J Am Mosq Control Assoc*. 2006;22(1):149–151.

Romito G, Pane E, Guglielmini C, Poser H, Valente C, Paradies P, Castagna P, Mazzoldi C, Cipone M. Efficacy and tolerability of the American Heartworm Society therapeutic protocol in dogs affected by heartworm disease without caval syndrome. *J Small Anim Pract*. 2023;1–8.

Rossi MID, Paiva J, Bendas A, et al. Effects of doxycycline on the endosymbiont *Wolbachia* in *Dirofilaria immitis* (Leidy, 1856)—Naturally infected dogs. *Vet Parasitol*. 2010;174:119-123.

Sarasola P, Jernigan AD, Walker DK, Castledine J, Smith DG, Rowan TG. Pharmacokinetics of selamectin following intravenous, oral and topical administration in cats and dogs. *J Vet Pharmacol Ther*. 2002;25(4):265–72.

Savadelis MD, Coleman AE, Rapoport GS, Sharma A, Sakamoto K, Keys DA, Ohmes CM, Hostetler JA, Dzimianski MT, Moorhead AR. Clinical assessment of heartworm-infected Beagles treated with a combination of imidacloprid/moxidectin and doxycycline, or untreated. *J Vet Intern Med*. 2020;34(5):1734-1745.

Savadelis MD, Day KM, Bradner JL, Wolstenholme AJ, Dzimianski MT, Moorhead AR. Efficacy and side effects of doxycycline versus minocycline in the three-dose melarsomine canine adulticidal heartworm treatment protocol. *Parasit Vectors*. 2018;11(1):671.

Savadelis MD, Ohmes CM, Hostetler JA, et al. Assessment of parasitological findings in heartworm-infected beagles treated with Advantage Multi® for dogs (10% imidacloprid + 2.5% moxidectin) and doxycycline. *Parasit Vectors*. 2017;10:245

Schnyder M. Deplazes P. Cross-reactions of sera from dogs infected with *Angiostrongylus vasorum* in commercially available *Dirofilaria immitis* test kits. *Parasit Vectors*. 2012;5:258.

Schulz BS, Hupfauer S, Ammer H, Sauter-Louis C, Hartmann K. Suspected side effects of doxycycline use in dogs–a retrospective study of 386 cases. *Vet Rec*. 2011;169:229.

Scoles GA, Dickson SL. New foci of canine heartworm associated with introductions of new vector species. *Proceedings of the Heartworm Symposium '95*, Auburn, AL. American Heartworm Society. 1995;27-35

Scoles GA, Dickson SL, Blackmore MS. Assessment of *Aedes sierrensis* as a vector of canine heartworm in Utah using a new technique for determining the infectivity rate. *J Am Mosq Control Assoc*. 1993;9:88-90.

Slocombe J, Srivastava B, Surgeoner G. The transmission period for heartworm in Canada. In *Proceedings of the Heartworm Symposium '95*, Auburn, AL. American Heartworm Society, 1995, pp 43–48.

Slocombe JOD, Surgeoner GA, Srivastava B. 1989. Determination of the heartworm transmission period and its used in diagnosis and control. In *Proceedings of the Heartworm Symposium '89*, Charleston, SC. American Heartworm Society, 1989, pp 19-26.

Sobotyk C, Savadelis MD, Verocai GG. Detection and cross-reaction of *Dirofilaria repens* using a commercial heartworm antigen test kit. *Vet Parasitol*. 2021;289:109302.

Sutton RH. Pathology and pathogenesis of dirofilariasis. In Boreham PFL and Atwell RB (Eds): *Dirofilariasis*. Boca Raton, FL: CRC Press, 1988, pp 99-132.

Tarbox AK, Swaroop M. Pulmonary embolism. *Int J Crit Illn Inj Sci*. 2013;3(1):69-72.

Taylor AE. The development of *Dirofilaria immitis* in the mosquito *Aedes aegypti*. *J Helminthol*. 1960;34:27-38.

Taylor MJ, Bandi C, Hoerauf A. *Wolbachia* bacterial endosymbionts of filarial nematodes. *Adv Parasitol*. 2005;60:245-284.

Terrell S. Heartworm in Alaska: Prevalence in domestic dogs and wild canids. In *Recent Advances in Heartworm Disease Symposium '98*, Tampa, FL. American Heartworm Society, 1998, pp 83-86.

Tjostheim SS, Kellihan HB, Grint KA, Stepien RL. Effect of sildenafil and pimobendan on intracardiac heartworm infections in four dogs. *J Vet Cardiol*. 2019;23:96-103.

Townson S, Tagboto S, McGarry HF, Egerton GL, Taylor MJ. *Onchocerca* parasites and *Wolbachia* endosymbionts: evaluation of a spectrum of antibiotic types for activity against *Onchocerca gutturosa* in vitro. *Filaria J*. 2006;5:4.

Vatta AF, Dzimianski M, Storey BE, Camus MS, Moorhead AR, Kaplan RM, Wolstenholme AJ. Ivermectin-dependent attachment of neutrophils and peripheral blood mononuclear cells to *Dirofilaria immitis* microfilariae in vitro. *Vet Parasitol*. 2014;206:38-42.

Velasquez L, Blagburn BL, Duncan-Decoq R, Johnson EM, Allen KE, Meinkoth J, Gruntmeir J, Little SE. Increased prevalence of *Dirofilaria immitis* antigen in canine samples after heat treatment. *Vet Parasitol*. 2014;206:67-70.

Venco L. Diagnosis of vena cava syndrome. *Veterinaria*. 1993;7:11-18.

Venco L, Genchi C, Vigevani Colson P, Kramer L. Relative utility of echocardiography, radiography, serologic testing and microfilariae counts to predict adult worm burden in dogs naturally infected with heartworms. In *Recent Advances in Heartworm Disease Symposium '01.* American Heartworm Society, 2001, pp 111-124.

Venco L, Manzocchi S, Genchi M, Kramer L. Heat treatment and false-positive heartworm antigen testing in ex vivo parasites and dogs naturally infected by *Dirofilaria repens* and *Angiostrongylus vasorum*. *Parasit Vectors*. 2017;10(Suppl 2):476.

Venco L, McCall JW, Guerrero J, Genchi C. Efficacy of long-term monthly administration of ivermectin on the progress of naturally acquired heartworm infections in dogs. *Vet Parasitol*. 2004;124:259-268.

Venco L, Mihaylova L, Boon JA. Right Pulmonary Artery Distensibility Index (RPAD Index). A field study of an echocardiographic method to detect early development of pulmonary hypertension and its severity even in the absence of regurgitant jets for Doppler evaluation in heartworm-infected dogs. *Vet Parasitol*. 2014;206(1-2):60-6.

Vezzoni A, Genchi C, Raynaud JP. Adulticide efficacy of RM 340 in dogs with mild and severe natural infections. In *Proceedings of the Heartworm Symposium '92*. Austin, TX. American Heartworm Society, 1992, pp 231-240.

Vila J, Alost E. Management and outcome of intracardiac heartworms in dogs. *Parasit Vectors*. 2023;16(1):146.

Wang LC. Comparison of a whole-blood agglutination test and an ELISA for the detection of the antigens of *Dirofilaria immitis* in dogs. *Ann Trop Med Parasitol*. 1998;92:73-77.

Weil GJ. *Dirofilaria immitis*: Identification and partial characterization of parasite antigens in the serum of infected dogs. *Exp Parasitol*. 1987;64:244-251.

Weil GJ, Malane MS, Powers KG, Blair LS. Monoclonal antibodies to parasite antigens found in the serum of *Dirofilaria immitis*-infected dogs. *J Immunol*. 1985;134(2):1185-1191.

Yoon WK, Choi R, Lee SG, Hyun C. Comparison of 2 retrieval devices for heartworm removal in 52 dogs with heavy worm burden. *J Vet Intern Med*. 2013;27(3):469-473.

AMERICAN HEARTWORM SOCIETY
EST. 1974

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.