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Literature review about the use of macrocyclic lactones in heartworm disease

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2024

Abstract

Heartworm disease, caused by *Dirofilaria immitis*, is an increasingly prevalent illness in canines that can lead to severe complications if left untreated. Transmitted by mosquitoes, it is a globally disseminated disease, particularly common in Europe, Asia, Australia, and North America. The objective of this review is to provide an overview of heartworm disease and emphasize the significance of Macrocyclic lactones in heartworm prophylaxis. Additionally, current findings on resistance, dosing strategies, and side effects are summarized. Macrocyclic lactones, which are the primary agents for heartworm prevention, are classified into two groups: avermectin and milbemycin. These compounds are especially effective against *Dirofilaria immitis* in its L3 and L4 larval stages. For optimal prophylaxis, preventive treatments are administered at 30-day intervals. Side effects associated with macrocyclic lactones are exceptionally rare, typically occurring in cases of overdose, intoxication, or in dogs with a multi-drug-resistant gene defect. A growing concern, however, is the emergence of macrocyclic lactone-resistant strains. Despite this, ivermectin, milbemycin oxime, and moxidectin continue to demonstrate excellent efficacy.

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1 Introduction

Dirofilaria immitis causes heartworm disease in dogs, distributed worldwide. Dirofilaria was first discovered by the Italian Francesco Birago in 1626, not as *D. immitis*, but as another parasite, *Dioctophyme renale*. Later in the 19th century, *D. immitis* received its name from an American parasitologist and was renamed in the 20th century by a French parasitologist.[1] *D. immitis* is transmitted through mosquitoes and is more prevalent in warmer regions. Mosquitoes ingest the microfilariae while taking blood from infected dogs. The microfilariae develop inside the mosquito and are transmitted to another dog during the next blood meal. At this point, the microfilariae develop inside the host and mature into adult worms. *D. immitis* migrates through the cardiovascular system until it reaches the heart of the dog, where it can lead to severe cardiac and pulmonary complications if not recognized and treated early. Adult worms have a long life span and can live up to seven years in the host. They can be found in dogs and cats; in rare circumstances, humans can become an accidental host.

Macrocyclic lactones (ML) are the primary class of drugs used to prevent *D. immitis* in dogs. This raises the question of how to use MLs properly to achieve the highest effectiveness in prophylaxis and treating heartworm disease in dogs. Not only are the usage and effectiveness fundamental, but also whether there are side effects or possible ML-resistant *D. immitis* strains are questions that should be considered.

The thesis will provide a literature review about using MLs in heartworm disease. The thesis is structured as follows: First, a general overview of heartworm disease is given. This includes all relevant facts about *D. immitis*, its characteristics, the life cycle, and a summary of *Dirofilaria repens*. Next, the clinical symptoms and prevalence of heartworm infection will be discussed. MLs constitute the next part of the thesis, starting with the active substances and properties of ivermectin, milbemycin oxime, moxidectin, selamectin, eprinomectin, and abamectin along with their mechanism of action. The usage of MLs in heartworm disease is another point of content, containing the measures and proceedings for *D. immitis* prophylaxis, diagnosis of heartworm disease, and the treatment options in case of positive diagnostic test results. Following the usage, the side effects of ML should be mentioned. This point is subdivided into a summary of dogs with a multi-drug-resistant gene

[1] Emerging Infectious diseases (2014) Etymologia: Dirofilaria. https://pmc.ncbi.nlm.nih.gov/articles/PMC3901479/ Accessed 23.07.2024

defect, toxic dosages and their clinical sign, and treatment in case of ML intoxication. The last point in this thesis implies the resistance of MLs against heartworm disease. The focus here is the spread of resistance, mechanism of action, detection of ML resistance, and the efficacy of different MLs against resistant strains.

2 Heartworm disease

2.1 Dirofilaria immitis

D. immitis is a parasitic nematode belonging to the superfamily Filarioidae, which is divided into three families: Filaridiae, Setariidae, and Onchocercidae. It is accountable for heartworm disease in canines and pulmonary or cutaneous infection in humans. Therefore, this parasite has a zoonotic potential despite its relatively rare occurrence rate. *D. immitis* has global distribution and can be located primarily in warm and tropical regions in America, Africa, South Asia, Australia, and several European countries.[2]

The intermediate hosts of *D. immitis* are arthropods, especially mosquitoes, and vertebrates are the definitive hosts. A vector of *D. immitis* can be any mosquito species that grants development into the infectious larval stage L3. This compromises around 60 mosquito species; amongst others, the most common ones are *Aedes*, *Anopheles*, and *Culex* species. It is possible to locate developmental stages in the definitive host and the intermediate host, increasing the complexity of their lifecycle. The adult worms are obligate endoparasites using the lobar and main pulmonary arteries as their home. In a severe infestation, the worms can also be found in the right ventricle of the heart.[3]

When considering the size of the worms, it reveals that adult male worms are around 18 cm long, whereas adult females can reach around 30 cm. Their life expectancy in vertebrates is five to seven years. The female worms release microfilaria without a sheath into the blood and are ovoviviparous. The microfilariae are 250-300 micrometers and can circulate for about two years in the bloodstream.[4]

Mosquitoes ingest the microfilariae during a blood meal from an infected definite host. Inside the mosquito, the microfilariae transition from larval stage L1 to the infectious larval stage L3. During a blood meal from a vertebrate host, the infectious larva can be transmitted and develop in the tissue for a few days before migrating to the blood vessels. The larva development continues in the definitive host, and they grow into larval stage L4 and ultimately into the juvenile larval stage L5, which consists of adult female and male worms.

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2 Noack, Sandra; Harrington, John et al (2021) Heartworm disease – Overview, intervention, and industry perspective. International Journal for Parasitology: Drugs and Drug resistance 16: 1-25 (2-4)

3 Noack, Sandra; Harrington, John et al (2021) Heartworm disease – Overview, intervention, and industry perspective. International Journal for Parasitology: Drugs and Drug resistance 16: 1-25 (1-4)

4 Noack, Sandra; Harrington, John et al (2021) Heartworm disease – Overview, intervention, and industry perspective. International Journal for Parasitology: Drugs and Drug resistance 16: 1-25 (2)

They live and mate in the heart of the host and release microfilaria after six months. For *D. immitis*, bacterial endosymbionts are essential for survival. In this case, they depend on rickettsia-like endosymbiont *Wolbachia*.[5]

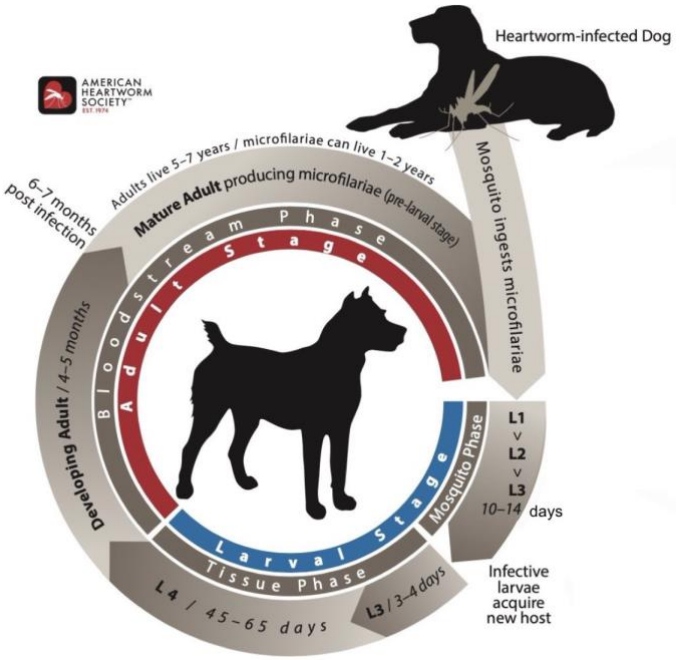

Figure 1 Lifecycle *D.immitis*[6]

2.2 Dirofilaria repens

D. repens is a widespread vector-borne disease and an evolving zoonosis in Europe. *D. immitis* and *D. repens* was long believed that they are the same parasite. It was only in 1910 that *D. repens* was officially named and distinct from *D. immitis*. *D. repens* belongs, as well as *D. immitis*, to the family Onchocercidae. Dogs are the main reservoir of *D. repens* infections, and microfilariae can be found in the blood. It has a prepatent period of about 170-238 days, and the life cycle consists of five larval stages. The larvae start to migrate through the subcutaneous tissue and muscular connective fasciae. In these parts, *D. repens* develop to the adult stage and stay throughout the rest of their life, which is about four years. The worms need an endosymbiont bacterium to survive long because they suppress the innate immunity of the host. Most of the time, the infection comes without clinical signs. Furthermore, no inflammatory reaction or connective tissue capsule can be seen around the

[5] Noack, Sandra; Harrington, John et al (2021) Heartworm disease – Overview, intervention, and industry perspective. International Journal for Parasitology: Drugs and Drug resistance 16: 1-25 (2-3)

[6] Nelson, Thomas C.; McCall, John W. et al Current Canine Guidelines for the Prevention, Diagnosis, and Management of Heartworm Infection in Dogs. American Heartworm Society 1-35 (6)

worm. In case of symptoms, pruritus, subcutaneous nodules, dermal swelling, and ocular conjunctivitis can be seen.[7]

2.3 Clinical Symptoms of heartworm disease

Heartworm disease may initially stay subclinical but regularly progresses to a clinical disease. Adult heartworms induce a vascular disease that develops to reduce blood flow, impacting the vasculature and the pulmonary system. In instances of extreme severity, it affects the right heart chambers.[8]

The clinical symptoms start with a mild persistent cough and unwillingness to exercise. Dogs affected by heartworm disease will also display a decreased appetite. Cardiac output will be reduced as a result of the impairment to the pulmonary endothelium and vascular blockage due to worm death. As a consequence, pulmonary hypertension develops, resulting in right-sided heart hypertrophy.[9] The following table (**Table 1**) summarizes the clinical signs according to the American Heartworm Society.[10]

Table 1 Clinical signs

Mild	Asymptomatic or cough
Moderate	Cough, exercise intolerance, abnormal lung sounds
Severe	Cough, exercise intolerance, abnormal lung and heart sounds, enlarged liver, syncope, ascites
Caval syndrome	Severe lethargy and weakness accompanied by hemoglobinemia and hemoglobinuria

Sporadically, enormous numbers of adult worms in the pulmonary arteries can impair the blood flow through the lungs. If the worms then shift to the right ventricle and the vena cava, the dog will die due to heart failure. This stage is also referred to as caval syndrome.[11]

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7 Genchi, Claudio; Kramer Laura (2016) Subcutaneous dirofilariosis (Dirofilaria repens): an infection spreading throughout the old world. Parasite & Vectors 10: 1-6 (1-2)

8 Noack, Sandra; Harrington, John et al (2021) Heartworm disease – Overview, intervention, and industry perspective. International Journal for Parasitology: Drugs and Drug resistance 16: 1-25 (4)

9 Noack, Sandra; Harrington, John et al (2021) Heartworm disease – Overview, intervention, and industry perspective. International Journal for Parasitology: Drugs and Drug resistance 16: 1-25 (4)

10 Nelson, Thomas C.; McCall, John W. et al Current Canine Guidelines for the Prevention, Diagnosis, and Management of Heartworm Infection in Dogs. American Heartworm Society 1-35 (21)

11 Prichard, Roger K. (2021) Macrocyclic lactone resistance in Dirofilaria immitis: risks for prevention of heartworm disease. Internal Journal for Parasitology 51: 1-12 (2)

2.4 Prevalence

D. immitis infections, mostly in tropical and warmer regions, can be found worldwide. On the one hand, heartworm infections increase in some areas due to climate change, but on the other hand, they decrease in a few countries because of more intensive control and better prevalence. The reduction of *D. immitis* occurs mainly in the Mediterranean due to declining mosquito populations, which could be due to industrialization, metropolitan expansion, and the use of insecticides.[12]

A closer look at *D. immitis* infection in Europe indicates that infection reduction is observed in Northern Italy and the Canary Islands. Due to climate changes, an increase is reported in Central and Northern Europe, including Finland, Estonia, and Serbia, which are located far to the north. The migration from heartworm-positive dogs to currently free countries also contributes to the spread of *D. immitis*. Heartworm disease can be found all over Australia, especially in the northern parts, but at the moment, occurrence rates are low. In Asia, *D. immitis* can be found, too; in China, the prevalence is 2% to 15%, and a new species was detected, which can also be found in India. A reduction of dirofilariosis was reported in Japan during the last few years. *D. immitis* was reported in Africa, but the information is limited. The following figure (**Figure 2**) provides an overview.[13]

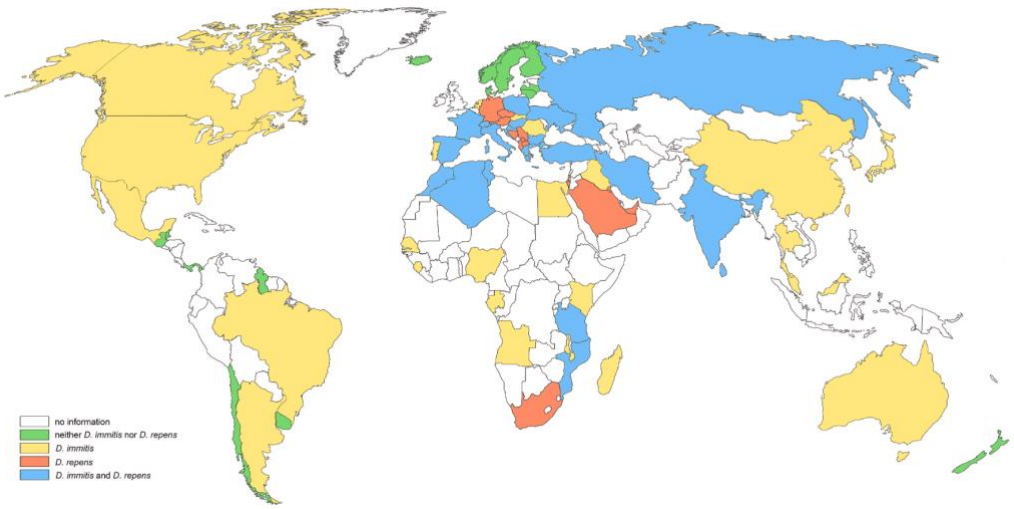

Figure 2 *D.immitis* and *D.repens* spread[14]

[12] Noack, Sandra; Harrington, John et al (2021) Heartworm disease – Overview, intervention, and industry perspective. International Journal for Parasitology: Drugs and Drug resistance 16: 1-25 (5)

[13] Noack, Sandra; Harrington, John et al (2021) Heartworm disease – Overview, intervention, and industry perspective. International Journal for Parasitology: Drugs and Drug resistance 16: 1-25 (5-6)

[14] Noack, Sandra; Harrington, John et al (2021) Heartworm disease – Overview, intervention, and industry perspective. International Journal for Parasitology: Drugs and Drug resistance 16: 1-25 (5)

Taking a closer look at the USA, it is conspicuous that *D. immitis* can be found all over the Americas. In the southern regions, prevalence is at 28%, and in the Gulf Coast regions, it reaches 48%. A survey conducted by the American Heartworm Society in 2019 showed that Mississippi, South Carolina, Louisiana, Alabama, and Arkansas have the highest incidence (**Figure 3**).[15]

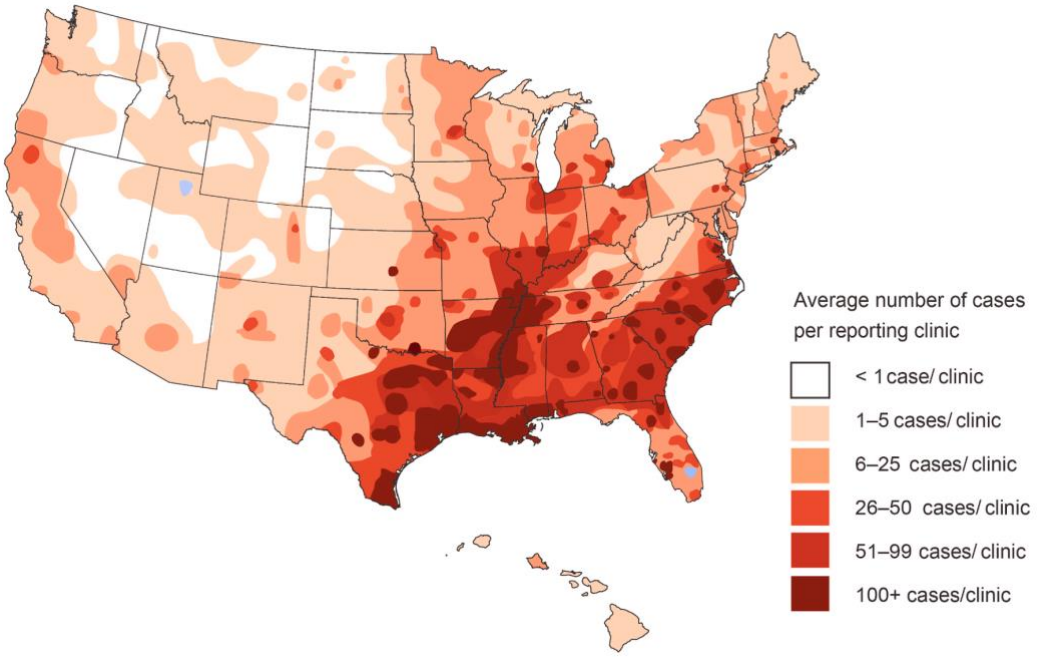

Figure 3 *D. immitis* cases in America[16]

[15] Noack, Sandra; Harrington, John et al (2021) Heartworm disease – Overview, intervention, and industry perspective. International Journal for Parasitology: Drugs and Drug resistance 16: 1-25 (5-6)

[16] Noack, Sandra; Harrington, John et al (2021) Heartworm disease – Overview, intervention, and industry perspective. International Journal for Parasitology: Drugs and Drug resistance 16: 1-25 (6)

3 Macrocyclic lactones

A closer look at the available heartworm preventives on the market demonstrates that they belong to the ML class of drugs.

3.1 Active substances

MLs are categorized into two distinct groups: the avermectins and the milbemycins. The avermectins cover abamectin, ivermectin, eprinomectin, and selamectin, whereas milbemycin oxime and moxidectin belong to the milbemycin. A 16-member ML ring is present in each of them, but the structural difference between those groups is that the avermectins have sugar residues at C13 of the macrocyclic ring while the milbemycins are protonated at C13.[17]

3.1.1 Ivermectin

Ivermectin is a chemically altered dihydro derivate of avermectin B1, consisting of over 80% 22,23-dihydro-avermectin B1a and under 20% 22,23 dihydro-avermectin B1b. It demonstrates efficacy against parasitic nematodes, arthropods such as flies, lice, and ticks, and against microfilariae L3 and L4 stages. Ivermectin reduces the fertility of adult worms, although it is not efficacious against them. Administered as oral, topical, or injectable preparations, it is generally considered safe but not utterly devoid of toxicity.[18]

3.1.2 Milbemycin oxime

Milbemycin oxime, belonging to the category of milbemycins, can be used for oral heartworm prevention. It comprises a mixture of 70-80% milbemycin A4 oxime and 20-30% milbemycin A3 oxime. Furthermore, it is effective against the immature and adult stages of roundworms, hookworms, whipworms, lungworms, and mites.[19]

3.1.3 Moxidectin

Moxidectin can be given orally, applied topically, or injected for heartworm prevention. Because of its high lipophilicity and long half-life, it can be used as a long-acting injection

[17] Noack, Sandra; Harrington, John et al (2021) Heartworm disease – Overview, intervention, and industry perspective. International Journal for Parasitology: Drugs and Drug resistance 16: 1-25 (8)

[18] Noack, Sandra; Harrington, John et al (2021) Heartworm disease – Overview, intervention, and industry perspective. International Journal for Parasitology: Drugs and Drug resistance 16: 1-25 (8)

[19] Noack, Sandra; Harrington, John et al (2021) Heartworm disease – Overview, intervention, and industry perspective. International Journal for Parasitology: Drugs and Drug resistance 16: 1-25 (8)

in dogs. The Food and Drug Administration has approved topical moxidectin for treating and removing microfilariae in dogs.[20]

3.1.4 Selamectin

Selamectin, another substance of the avermectin group, is a semisynthetic monosaccharide oxime derivate of doramectin, the most potent nematicide of the avermectins. As a topical formulation, selamectin can be purchased for dogs and cats and is effective against *Dirofilaria immitis*, fleas, ticks, and gastrointestinal nematodes.[21]

3.1.5 Eprinomectin

Eprinomectin, a semi-synthetical derivate of avermectin B1, was precisely created for veterinary medicine and used for cattle, including lactating animals. As a semi-synthetic derivate of avermectin B1, it is used for cats as a topical endectoparasiticide in combination with fipronil (S)-methoprene and praziquantel.[22]

3.1.6 Abamectin

Abamectin is the only ML used for animal health and crop protection. It is a semi-synthetic product of avermectin B1 and is utilized primarily in Australia as heartworm prevention in combination with oxibendazole and praziquantel.[23]

3.2 Mechanism of action

MLs are highly effective allosteric agonists of the nematode's Glutamate-gated chloride channels (GluCl). A range of GluCl sequences within the nematode makes these channels sensitive and insensitive to MLs. The channels are part of a superfamily of cys-loop ligand-ligated ion channels, and their structure is defined by a loop formed by amino acids, which are sealed by disulfide-bonded cysteine residues.[24]

Nematodes and arthropods, along with filariae, have these GluCl channels. MLs can alter the GluCl channels in vertebrates and other ion channel groups in vertebrates and invertebrates. GluCl channels are the most significant for treating heartworm disease. Due

[20] Noack, Sandra; Harrington, John et al (2021) Heartworm disease – Overview, intervention, and industry perspective. International Journal for Parasitology: Drugs and Drug resistance 16: 1-25 (9)

[21] Noack, Sandra; Harrington, John et al (2021) Heartworm disease – Overview, intervention, and industry perspective. International Journal for Parasitology: Drugs and Drug resistance 16: 1-25 (8)

[22] Noack, Sandra; Harrington, John et al (2021) Heartworm disease – Overview, intervention, and industry perspective. International Journal for Parasitology: Drugs and Drug resistance 16: 1-25 (8)

[23] Noack, Sandra; Harrington, John et al (2021) Heartworm disease – Overview, intervention, and industry perspective. International Journal for Parasitology: Drugs and Drug resistance 16: 1-25 (8)

[24] Noack, Sandra; Harrington, John et al (2021) Heartworm disease – Overview, intervention, and industry perspective. International Journal for Parasitology: Drugs and Drug resistance 16: 1-25 (10)

to differences in subunit sequences, structural differences in the ML binding site can be seen. This results in variable sensitivities and the response of the ligand-gated chloride channel to MLs.[25]

GluCl channels are found in the parasites` nervous system and pharyngeal muscles. MLs induce paralysis, resulting in a decrease in nutrient intake, removal of worms from their predilection side, and a decrease in egg production. Infertility due to MLs is linked to male worms.[26]

In dogs, MLs bind to γ-aminobutyric acid type A-gated chloride channels in the central nervous tissue. Blood-brain-barrier (BBB) prevents MLs from binding. P-glycoprotein (P-gp) transports the absorbed substrates back into the body and restricts the entry of MLs into the central nervous system (CNS).[27]

[25] Noack, Sandra; Harrington, John et al (2021) Heartworm disease – Overview, intervention, and industry perspective. International Journal for Parasitology: Drugs and Drug resistance 16: 1-25 (11)

[26] Wolstenholme Adrian J.; Evans Christopher C. et al (2015) The emergence of macrocyclic lactone resistance in the canine heartworm, Dirofilaria immitis. Cambridge University Press: 1-11 (4)

[27] Merola, Valentina M, Eubig, Paul A. (2018) Toxicology of Avermectins and Milbemycins (Macrocyclic lactones) and the Role of P-Glycoprotein in Dogs and Cats. Vet Clin Small Anim 48: 1-22 (2)

4 Usage of macrocyclic lactones in heartworm disease

4.1 Prophylaxis of heartworm disease

The prophylaxis of heartworm disease in dogs is of high importance. Due to the morbidity and mortality caused by adult worms, preventing the manifestation of the adult stage is crucial. To decrease the risk of mosquito bites. Insect repellents and mosquito abatement can be used. Nevertheless, preventing dogs from getting bitten by mosquitoes is challenging.[28]

As mentioned, MLs are the currently available heartworm preventives on the market. They are very effective in combating L3 and L4 infection in dogs. Milbemycin oxime and ivermectin can be used for oral administration once a month. Moxidectin and selamectin are used monthly as well but applied topically. Moxidectin can be used parenterally as a single dose of slow-release formulation and protects for 6 months.[29]

The American Heartworm Society recommends a year-round heartworm prophylaxis to ensure optimal protection. In Europe, prevention only during the mosquito season is also common. Nevertheless, the period should be extended by 1-2 months before and after the season, depending on the product type used. Heartworm prevention is administered at a 30-day interval due to the time it takes for L3 to develop into L4 and the high sensitivity of L4 to MLs. Furthermore, the long half-life of MLs plays a role.[30]

4.2 Diagnosis

A yearly screening for all dogs is recommended by the American Heartworm Society, including an antigen test and a microfilaria test to achieve optimal prophylaxis effectiveness and avoid infection.

The antigen available tests are enzyme-linked immunosorbent assay (ELISA) and immunochromatographic tests. The tests can detect the protein secreted by adult female worms, which means at least one mature female worm needs to be present for a positive result. A positive antigen test shows a specific heartworm antigen, but sometimes the test can be false-positive, too. Therefore, the dogs should be tested further before starting the treatment. In the case of false-negative tests, the infection could be mild, the female

[28] Prichard, Roger K. (2021) Macrocyclic lactone resistance in Dirofilaria immitis: risks for prevention of heartworm disease. Internal Journal for Parasitology 51: 1-12 (3)

[29] Prichard, Roger K. (2021) Macrocyclic lactone resistance in Dirofilaria immitis: risks for prevention of heartworm disease. Internal Journal for Parasitology 51: 1-12 (3)

[30] Prichard, Roger K. (2021) Macrocyclic lactone resistance in Dirofilaria immitis: risks for prevention of heartworm disease. Internal Journal for Parasitology 51: 1-12 (3)

immature, or only male worms are present. It should be clear that a negative antigen test does not imply that there is no heartworm infection but only that no antigens are present.[31]

A modified Knott test is used to detect microfilariae. In this regard, EDTA blood is mixed with formalin, and methylene blue is added after receiving sediment. The blood is then placed on a slide and examined under the microscope for microfilariae. Testing dogs for microfilariae is always necessary because if high numbers of microfilariae are present, severe reactions can follow after starting the treatment.[32]

Other diagnostic aids include radiography and echocardiography. Radiography helps to determine whether cardiopulmonary diseases are present. Alteration of the heart due to heartworm disease could be pulmonary arterial changes or, in severe cases, an enlargement of the right heart. Echocardiography can show heartworms in the pulmonary artery, interlobar branches, or the right heart. An impaired function of the heart due to the worm burden can also be detected.[33]

In circumstances of noncompliance or when changing products, the dog should be tested for antigens and microfilariae again. Following noncompliance, three tests in the first year are performed, and annual testing is advised (**Figure 4**).[34]

Figure 4 Testing protocol following noncompliance[35]

4.3 Treatment of infected animals

The goal of treating heartworm infection is to eradicate all life stages of the heartworm disease, including microfilariae, larval stages, juvenile and adult worms. Besides removing the worms, improving the clinical symptoms of the dogs and minimizing the post-treatment complications is extremely important. The American Heartworm Society, therefore,

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31 Nelson, Thomas C.; McCall, John W. et al Current Canine Guidelines for the Prevention, Diagnosis, and Management of Heartworm Infection in Dogs. American Heartworm Society 1-35 (15)

32 Nelson, Thomas C.; McCall, John W. et al Current Canine Guidelines for the Prevention, Diagnosis, and Management of Heartworm Infection in Dogs. American Heartworm Society 1-35 (15-16)

33 Nelson, Thomas C.; McCall, John W. et al Current Canine Guidelines for the Prevention, Diagnosis, and Management of Heartworm Infection in Dogs. American Heartworm Society 1-35 (17-18)

34 Nelson, Thomas C.; McCall, John W. et al Current Canine Guidelines for the Prevention, Diagnosis, and Management of Heartworm Infection in Dogs. American Heartworm Society 1-35 (16-17)

35 Nelson, Thomas C.; McCall, John W. et al Current Canine Guidelines for the Prevention, Diagnosis, and Management of Heartworm Infection in Dogs. American Heartworm Society 1-35 (17)

recommends glucocorticoids, vasodilators, positive inotropic agents, diuretics, and fluid therapy.[36]

Melarsomine dihydrochloride is the only approved drug for treating adulticide heartworms. Unfortunately, the drug comes along with severe pulmonary thrombosis and is not allowed in every country. Several studies show an advantage of MLs, together with doxycycline, before the administration of melarsomine. Pre-treatment may diminish worm bulk and eradicate pro-inflammatory antigens, which decreases post-adulticide complications.[37]

Doxycycline helps remove *Wolbachia spp.* bacteria, which is required for parasite development, fertility, and survival. It significantly impedes the development of heartworms, but its adulticidal activity is limited.[38]

It has previously been shown that MLs are efficient against L3 and L4. The preventive dose of MLs has a slow-kill effect on adult heartworms. Research indicates that among dogs affected with *Dirofilaria immitis*, 100% were negative for circulating microfilariae after four months of monthly ivermectin prophylaxis, while 71% were negative for circulating antigens after 24 monthly doses. A disadvantage is that interstitial inflammation and pulmonary hypertension rise by 20%. In addition, prolonged use of MLs in dogs with heartworm disease has been associated with the emergence of resistant *Dirofilaria immitis* strains.[39]

Several studies with ivermectin demonstrated significant efficacy and safety during monthly administration at the minimum dosage of 6 μg/kg. If the interval of 30 days is prolonged, the efficiency is still preserved. A closer look at the adult heartworms that survive the monthly ivermectin dosage shows that they decrease in size and are less agile. Furthermore, the intestinal epithelium transforms after one year of monthly administration if the treatment starts when the heartworms are five months old. Monthly ivermectin has a particular drug effect on three to eight-month-old heartworms. An early-initiated treatment increases the number of worms that die. In addition, the chances of developing a patent infection are lower. The later the treatment starts, the longer the heartworm survives, which means it is more

[36] Nelson, Thomas C.; McCall, John W. et al Current Canine Guidelines for the Prevention, Diagnosis, and Management of Heartworm Infection in Dogs. American Heartworm Society 1-35 (20)

[37] Kramer, L; Crosara, S et al (2018) Wolbachia, doxycycline and macrocyclic lactones: New prospects in the treatment of canine heartworm disease. Veterinary Parasitology 254: 1-3 (2)

[38] Kramer, L; Crosara, S et al (2018) Wolbachia, doxycycline and macrocyclic lactones: New prospects in the treatment of canine heartworm disease. Veterinary Parasitology 254: 1-3 (2)

[39] Kramer, L; Crosara, S et al (2018) Wolbachia, doxycycline and macrocyclic lactones: New prospects in the treatment of canine heartworm disease. Veterinary Parasitology 254: 1-3 (2-3)

probable for the antigens to be detected. Consequently, the dog will be longer antigen positive.[40]

Studies showed that in terms of its adulticide activities, milbemycin oxime has been shown not as effective as ivermectin when administered monthly. After an administration for 13 months against three-month-old heartworms, their effectiveness was high and equal. Moderate success (41.1%) was demonstrated when milbemycin oxime was administered for one year against four-month-old heartworms, whereas ivermectin was highly successful (95.1%). After being given for 16 months against eight-month-old worms, milbemycin oxime was ineffective, and ivermectin, in combination with pyrantel pamoate, killed 56%.[41]

With moxidectin, a high efficiency (85.9%) was demonstrated by a single treatment with the injectable, sustained-release formulation against four months old heartworms. After a second injection six months later, the effectiveness was much more elevated (97.2%). In contrast, the success decreased to 25% when administered against six months old heartworms. Given orally, moxidectin has a 100% efficacy against two months old heartworms.[42]

Selamectin shows 100% success against two-month-old heartworms; when administered for 1 year, a 98.5% effectiveness against three-month-old worms is reported.[43]

The American Heartworm Society recommends using ivermectin in their treatment protocol, and most of the studies were conducted with this ML. That is why the Small Animal Clinic of the University of Veterinary Medicine Budapest published a study with 44 dogs using moxidectin as ML during the treatment with melarsomine. Before starting the treatment, the dogs were tested for microfilariae with Knott tests and antigens with VetScan antigen test. PCR was used to distinguish *D. immitis* and *D. repens*. The dogs were grouped into classes, depending on their clinical signs and thorax radiographs, starting with class 1 (mild) to class 4 (most severe). In classes 3 and 4, dogs with clinical signs were treated symptomatically before starting the treatment protocol. Dogs with microfilariae were under supervision after the administration of moxidectin. The following treatment was conducted as the American Heartworm Society recommended, as shown in **Table 2**. Results showed that in two of seven

[40] McCall, John W. (2005) The safety-net story about macrocyclic lactone heartworm preventives: A review, an update, and recommendations. Veterinary Parasitology 133: 1-10 (2-4)

[41] McCall, John W. (2005) The safety-net story about macrocyclic lactone heartworm preventives: A review, an update, and recommendations. Veterinary Parasitology 133: 1-10 (5)

[42] McCall, John W. (2005) The safety-net story about macrocyclic lactone heartworm preventives: A review, an update, and recommendations. Veterinary Parasitology 133: 1-10 (5-6)

[43] McCall, John W. (2005) The safety-net story about macrocyclic lactone heartworm preventives: A review, an update, and recommendations. Veterinary Parasitology 133: 1-10 (6)

dogs in class 3, heartworms diminished from the main pulmonary artery six and ten days after the first moxidectin application. Furthermore, no anaphylactic reactions have developed, showing that moxidectin is a safe and efficient ML. Nevertheless, an anaphylactic reaction because of moxidectin can happen, and a pre-treatment with prednisolone and clopidogrel for microfilaremic patients is advised. Antihistamine and glucocorticoids should also be given together with the first application of moxidectin, and it is also suggested in the case of ivermectin.[44]

In the following table (**Table 2**), a summary of the American Heartworm Society treatment protocol can be seen:[45]

Table 2 Treatment protocol according to the American Heartworm Society

Day	**Treatment**
Day 0	Dogs are diagnosed and confirmed as Heartworm-positive. A strict exercise restriction is recommended, and dogs with clinical symptoms should be stabilized.
Day 1	Administer of heartworm prevention.
Day 1-28	Administer of doxycycline 10 mg/kg BID for 4 weeks.
Day 30	Administer of heartworm prevention.
Day 60	Administer heartworm prevention and first melarsomine injection of 2.5 mg/kg intramuscularly.
Day 90	Administer heartworm prevention and a second melarsomine injection of 2.5 mg/kg intramuscularly.
Day 91	Third melarsomine injection 2.5 mg/kg intramuscularly.
Day 120	Test for microfilaria; if it is positive, treat it with a microfilaricide and retest in four weeks. Continue heartworm prevention.

[44] Vörös, Károly; Becker Zsolt et al (2022) Application of Moxidectin and Ultrasound-Aided Injection of Melarsomine During the American Heartworm Society Recommended Treatment Protocol in Dirofilaria immitis Infected Dogs. Vectore-borne and zoonotic diseases 22: 1-9 (2-6)

[45] Nelson, Thomas C.; McCall, John W. et al Current Canine Guidelines for the Prevention, Diagnosis, and Management of Heartworm Infection in Dogs. American Heartworm Society 1-35 (25)

Day 365	Perform an antigen test nine months after the last melarsomine injection. If the antigen test is positive, the dog should be re-treated with doxycycline and two doses of melarsomine 24 hours apart.

In case of severe worm burden or caval syndrome, surgical extraction of adult heartworms is possible when worms block the dog's blood flow. If the worms are in the right atrium or the tricuspid valve, they can be removed with forceps through the right jugular vein under light sedation or local anesthesia.[46]

After starting the treatment against *D. immitis*, dogs with a high worm burden must be observed. The dog can be monitored from home or in a veterinary clinic. In addition, pre-treatment with antihistamines and corticosteroids can be used. In rare cases, dogs develop hypersensitive reactions like tachypnea, salivation, vomiting, or depression after the administration of MLs. Diphenhydramine, dexamethasone, and intravenous fluids can be used for treatment.[47]

Pneumonitis can develop due to the death of microfilariae or adult worms. Because of eosinophilic inflammations, crackles can be heard during auscultation and coughing. As a result of alveolar capillary or parabronchial artery injury caused by the worms, hemoptysis can appear. Dogs can be treated with corticosteroids and oxygen and rest at home or in the hospital. During heartworm therapy, dogs have an elevated inflammatory biomarker C-reactive protein and interleukin-6 level. Furthermore, increased D-dimer levels can be detected as a consequence of thrombosis. Living heartworms have anticoagulant properties, which means that dead worms contribute to heartworm pulmonary thromboembolism.[48]

[46] Nelson, Thomas C.; McCall, John W. et al Current Canine Guidelines for the Prevention, Diagnosis, and Management of Heartworm Infection in Dogs. American Heartworm Society 1-35 (24-27)

[47] Ames, Marisa K.; Atkins, Clarke E. (2020) Treatment of dogs with severe heartworm disease. Veterinary Parasitology 283: 1-6 (1-3)

[48] Ames, Marisa K.; Atkins, Clarke E. (2020) Treatment of dogs with severe heartworm disease. Veterinary Parasitology 283: 1-6 (3-5)

5 Side effects of macrocyclic lactones

MLs have a high margin of safety, and side effects are therefore rare. Nevertheless, side effects can appear in cases of overdose or dog breeds with a multi-drug-resistant (Mdr1) gene defect.

As explained in the mechanism of action, MLs bind to γ-aminobutyric acid type A-gated chloride channels in dogs. In case of an overdose, ML passes through the BBB. Thereby, hyperpolarization and reduced activity of excitatory neurons that express chloride channels are caused by a chloride influx and result in clinical signs. These signs can be CNS signs, including ataxia, mydriasis, blindness, hypersalivation, tremors, neurological depression, and seizures. Severe signs can also lead to a comatose state, and due to the long half-life of MLs, intoxication and, therefore, clinical signs can last for several days up to weeks, depending on the dog breed and dosage.[49]

5.1 Dogs with Mdr1 gene defect

In some dog breeds, especially collies and Shetland sheepdogs, whippets, windhounds, or German shepherds, a genetic defect in P-gp is present. As previously explained, it is in charge of limiting the entry of MLs into the CNS. In these dogs, P-gp is not effective anymore because of a four-base pair deletion in the Mdr1 gene. This leads to a buildup of P-gp substrates in the brain. Hereby, the BBB is impaired, and MLs can pass through, which leads to an accumulation in the CNS and neurological effects (**Figure 5**).[50]

[49] Merola, Valentina M, Eubig, Paul A. (2018) Toxicology of Avermectins and Milbemycins (Macrocyclic lactones) and the Role of P-Glycoprotein in Dogs and Cats. Vet Clin Small Anim 48: 1-22 (1-2)

[50] Merola, Valentina M, Eubig, Paul A. (2018) Toxicology of Avermectins and Milbemycins (Macrocyclic lactones) and the Role of P-Glycoprotein in Dogs and Cats. Vet Clin Small Anim 48: 1-22 (2-4)

5.2 Toxic dosages and clinical signs

Agent	Formulations	Therapeutic Dosages (Labeled and Off-Label, mg/kg)	Acute, Subacute, or Chronic Dosages Published as Safe (mg/kg)	Toxic Dosages ML Sensitive Dogs (mg/kg)	Acute Toxic Dosage Normal Dog/ Cat (mg/kg)
Ivermectin	Tablets, oral liquid, oral paste, feed premix, injectable, topical, otic	0.006–0.6 PO D 0.024 PO C 0.2–0.4 SC D, C	0.5 PO daily × 12 wk[a] D 0.06 PO Collies 0.2–1.33[a] PO or SC C 0.72 PO C	0.1–0.4[b] PO 0.2–0.25[b] SC	0.2–2.5 PO D 0.3 SC C
Selamectin	Topical	6 topical D, C	6 PO D, C[c] 40 topical Collies 72–114 topical D 236–367 topical C	5 PO[d]	None found
Moxidectin	Tablets, oral drench, injectable, topical	0.003 PO D 0.17 sustained-release SC D 2.5 topical D 1 topical C	1.15 PO daily × 1 y D 0.09 PO Collies 0.85 SC D, Collies	1 PO[e]	1.9–2.8 PO D 1 PO C[f]
Doramectin	Injectable, pour-on	0.6 SC D, C	0.5–1 PO daily × 91 d D 0.2 SC C	0.2[g]–0.7 SC	None found
Milbemycin	Tablets	0.5–2 PO D 2 PO C	10 PO Collies 10 PO C	5–10[g] PO 0.8 PO × 2 d 1.5 PO × 13 d	None found

Abbreviations: C, cat; collies, ivermectin-sensitive collies; D, dog; PO, orally; SC, subcutaneously.
[a] It should be noted that some animals are also reported to have problems at this dosage.
[b] Many of the collies in these reports were not tested for the *ABCB1-1Δ* gene defect.
[c] Cats exhibited drooling and intermittent vomiting with oral dosing.
[d] One collie was ataxic after this dosage in the safety studies, but others tolerated up to 15 mg/kg PO.
[e] Administered as a product containing 2.5% moxidectin and 10% imidacloprid.
[f] Generally only mild signs seen.
[g] Collies at these dosages were not tested for the *ABCB1-1Δ* gene defect.

Figure 1 Different ML dosages in normal and sensitive dogs[51]

The table above shows an overview of the therapeutic dose: nontoxic, toxic for ML sensitive, and toxic for normal dogs. For heartworm prevention, Ivermectin is used with a dosage of 0.006-0.012 mg/kg in dogs, and off label, 0.05-0.2 mg/kg against microfilariae and 0.03-0.06 mg/kg against ectoparasites. In dogs without a gene mutation, mild clinical signs can be seen at a dosage starting with 0.2 mg/kg, and dogs with an ML sensitivity of 0.06 mg/kg can be toxic. Clinical signs in an overdose of ivermectin can be lethargy, ataxia, tremor, mydriasis, hypersalivation, blindness, and bradycardia.[52]

Selamectin is used with a 6 mg/kg dosage as a topical spot-on. Therefore, overdoses are very unlikely. In case of clinical signs, vomiting, lethargy, licking of lips, ataxia, or anorexia are present.[53]

Moxidectin is used therapeutically at 0.003 mg/kg per os and 0.17 mg/kg as subcutaneous injections in dogs. The toxic dosage is 1 mg/kg for ML-sensitive dogs; otherwise, it is 1.9-2.8 mg/kg. Signs in dogs are the same as in ivermectin overdose, including, among other

[51] Merola, Valentina M, Eubig, Paul A. (2018) Toxicology of Avermectins and Milbemycins (Macrocyclic lactones) and the Role of P-Glycoprotein in Dogs and Cats. Vet Clin Small Anim 48: 1-22 (5)
[52] Merola, Valentina M, Eubig, Paul A. (2018) Toxicology of Avermectins and Milbemycins (Macrocyclic lactones) and the Role of P-Glycoprotein in Dogs and Cats. Vet Clin Small Anim 48: 1-22 (4)
[53] Merola, Valentina M, Eubig, Paul A. (2018) Toxicology of Avermectins and Milbemycins (Macrocyclic lactones) and the Role of P-Glycoprotein in Dogs and Cats. Vet Clin Small Anim 48: 1-22 (6-7)

things, ataxia, tremors, seizures, tachycardia, bradycardia, respiratory distress, or blindness.[54]

In dogs, doramectin is used with a dosage of 0.6 mg/kg and can be toxic when given at a dosage of 0.2-0.7 mg/kg to sensitive dogs. Clinical signs can be blindness, CNS depression, recumbency, head pressing, disorientation, or tremors.[55]

Milbemycin oxime is used in dogs for heartworm prevention at a dosage of 0.5-2 mg/kg. ML-sensitive dogs can develop clinical signs such as ataxia, hypersalivation, mydriasis, and lethargy if the dosage is 5-10 mg/kg.[56]

Taking a closer look at the therapeutic dosages and the dosages for ML intoxication, it is noticeable that in most cases, the dogs must consume a considerable amount of the therapeutic dose to result in a toxicosis. In most situations where ML overdose was reported, the dogs were treated or accidentally got the product labeled for large animals, which resulted in a wrong dosage.[57]

5.3 Treatment of ML intoxication

ML toxicosis does not have a specific antidote. Treatment of ML toxicosis is done with decontamination and supportive care. Induced emesis could be an option if the consumption were within the last 30 to 60 minutes, but it depends on various criteria. For example, the composition of the formulation. In the case of liquid or paste formulations, it is challenging to induce emesis because they are absorbed much faster than tablets. Another factor is whether the dog has eaten a meal before ingesting an overdose of ML because food retards the emptying of liquid formulations. If clinical signs have already appeared, caution when inducing emesis is vital. The danger of an aspiration in case of neurological signs is high. Activated charcoal can be given as another treatment option within four hours after the ingestion. During ivermectin toxicosis, activated charcoal should be administered every eight hours for two days. As in the case of induced emesis, activated charcoal should be used carefully if clinical signs or, in severe cases, seizures or coma are present. In the case of

[54] Merola, Valentina M, Eubig, Paul A. (2018) Toxicology of Avermectins and Milbemycins (Macrocyclic lactones) and the Role of P-Glycoprotein in Dogs and Cats. Vet Clin Small Anim 48: 1-22 (6)

[55] Merola, Valentina M, Eubig, Paul A. (2018) Toxicology of Avermectins and Milbemycins (Macrocyclic lactones) and the Role of P-Glycoprotein in Dogs and Cats. Vet Clin Small Anim 48: 1-22 (7-8)

[56] Merola, Valentina M, Eubig, Paul A. (2018) Toxicology of Avermectins and Milbemycins (Macrocyclic lactones) and the Role of P-Glycoprotein in Dogs and Cats. Vet Clin Small Anim 48: 1-22 (7)

[57] Merola, Valentina M, Eubig, Paul A. (2018) Toxicology of Avermectins and Milbemycins (Macrocyclic lactones) and the Role of P-Glycoprotein in Dogs and Cats. Vet Clin Small Anim 48: 1-22 (4)

supportive care, fluid therapy can be used. In addition, oxygen and intubation can be necessary, depending on the severity.[58]

[58] Merola, Valentina M, Eubig, Paul A. (2018) Toxicology of Avermectins and Milbemycins (Macrocyclic lactones) and the Role of P-Glycoprotein in Dogs and Cats. Vet Clin Small Anim 48: 1-22 (13-14)

6 Resistance of macrocyclic lactones against heartworm disease

6.1 Spread of resistance

ML resistance is mainly found in the USA, and records of failing ML preventives were first announced in 2005. In the beginning, the loss of effectiveness was expected to be due to failure of administration by the owner. The first study with evidence of failing MLs was released in 2011 and reported the ineffectiveness of ivermectin and milbemycin oxime administered regularly in increased dosages. ML-resistant strains are becoming increasingly prevalent in the lower half of the Mississippi River in the USA. There is a significant worry of spreading to other regions in North America.[59]

6.2 Mechanism of resistance

To understand the development of ML resistance, it is fundamental to take a step back to the mechanism of action. MLs target the glutamate-gated chloride channels at the neuromuscular junctions in nematodes. To reach a flaccid paralysis of the muscles in nematodes, ML opens the chloride channels due to the rise of chloride ion. They are not only acting on glutamate-gated chloride channels but also many other ligand-gated anion channels. Depending on the nematode, they act in different places. MLs paralyze the pharynx in hookworms, which inhibits the uptake of nutrients. They may also induce a paralytic effect, resulting in the parasite's physical removal. However, this is not always the case for filarial parasites. They can survive even with a paralyzed pharynx because they absorb nutrients through their cuticle.[60]

Another advantage of filarial nematodes is that they can survive with a paralyzed body for some time without being physically removed. ML reduces the fertility and reproduction of adult worms, which means that a single administration of MLs cannot kill many adult worms. Nonetheless, repeated treatment with MLs can shorten the lifespan of adult worms. If the resistance develops in filariae L3 and L4, they can evolve into adult worms that reproduce and transmit their genes, responsible for resistance, to the next generation of filariae. Eventually, the resistant filariae are then transmitted by vectors to other dogs.[61]

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[59] Prichard, Roger K. (2021) Macrocyclic lactone resistance in Dirofilaria immitis: risks for prevention of heartworm disease. Internal Journal for Parasitology 51: 1-12 (4-5)

[60] Prichard, Roger K. (2021) Macrocyclic lactone resistance in Dirofilaria immitis: risks for prevention of heartworm disease. Internal Journal for Parasitology 51: 1-12 (5-7)

[61] Prichard, Roger K. (2021) Macrocyclic lactone resistance in Dirofilaria immitis: risks for prevention of heartworm disease. Internal Journal for Parasitology 51: 1-12 (5-7)

Another factor that promotes ML resistance is inbreeding in *D. immitis*. Dogs diseased with heartworms only have a few adult worms. This means these male and female adult worms produced all microfilariae in the host. If a vector transmits the filariae to another dog, the chances are high that they are siblings or half-siblings. The filariae will evolve into adult worms and reproduce. In the case of resistant microfilariae, they will later produce even more ML-resistant larvae. Conclusively, inbreeding in *D. immitis* results in even more resistance.[62]

6.3 Detection of macrocyclic lactone resistance

Currently, no clinically acceptable test for detecting ML-resistant strain in *Dirofilaria immitis* exists. The gold standard procedure is to collect blood from infected animals and cultivate it via mosquitoes until it reaches the L3 stage. Afterward, given the proper dose of ML preventatives, experimental animals are infected with L3. Then, wait for the emergence of an infection. The process takes about eight months and is impractical for clinical use.[63]

An alternative is to utilize a surrogate measurement to determine the drug's effectiveness. Different studies demonstrate the decline of microfilaria in diseased dogs with identified ML-sensitive heartworms if treated with microfilaricidal doses. Within a few months under treatment, they transition to an amicrofilaremic state. This leads to the conclusion that a dog receiving monthly ML prophylaxis is *Dirofilaria immitis*-free until infected with a drug-resistant biotype. Conversely, if an ML resistance is confirmed, the dog was microfilaremic at the time of diagnosis despite monthly treatment. These implications help to evolve a clinical test. This test measures the microfilaria levels before and after a microfilaricidal dose of MLs. The test is called microfilarial suppression test and is the most practical method. It consists of three steps; the first one is the performance of Knott`s test for quantitation of microfilaria. Next, ivermectin (50 μg/kg) or milbemycin oxime (1mg/kg) is administered. Due to the risk of anaphylaxis, the dog should be hospitalized for six to eight hours after the administration. Finally, seven days after the first test, a second Knott test is performed. A reduced microfilaria >75% between both tests implies a low ML suspicion. In contrast, the suspicion is high if the number is <75% (**Figure 6**).[64]

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62 Prichard, Roger K. (2021) Macrocyclic lactone resistance in Dirofilaria immitis: risks for prevention of heartworm disease. Internal Journal for Parasitology 51: 1-12 (5-7)

63 Moorhead, Andrew R.; Evans, Christopher C. et al (2017) A diagnostic algorithm for evaluating cases of potential macrocyclic lactone-resistant heartworm. Parasite & Vectors 10: 1-5 (1-4)

64 Moorhead, Andrew R.; Evans, Christopher C. et al (2017) A diagnostic algorithm for evaluating cases of potential macrocyclic lactone-resistant heartworm. Parasite & Vectors 10: 1-5 (2-4)

The following illustration shows a decision tree for ML resistance cases. Starting with the positive heartworm antigen test, the next step is taking history and discovering if there are substantial gaps in the administration of heartworm prophylaxis. In case of treatment gaps of at least two months, the heartworm infection is not expected to result from an infection with a resistant biotype, and it should proceed with the American Heartworm Society recommended treatment protocol. When the resistance is still suspected because of no major gaps, microfilaria is observed. Resistance cannot be performed if no microfilariae are found. If the microfilaria observation is positive, a microfilaria suppression test is the next phase. If there is a decrease of >75%, the AHS protocol can be performed. In case of no such reduction of microfilaria, the AHS treatment protocol will proceed with an initiated doxycycline treatment at 10 mg/kg BID for 30 days and a topical mosquito repellent to avoid transmission of the possible resistant biotype.[65]

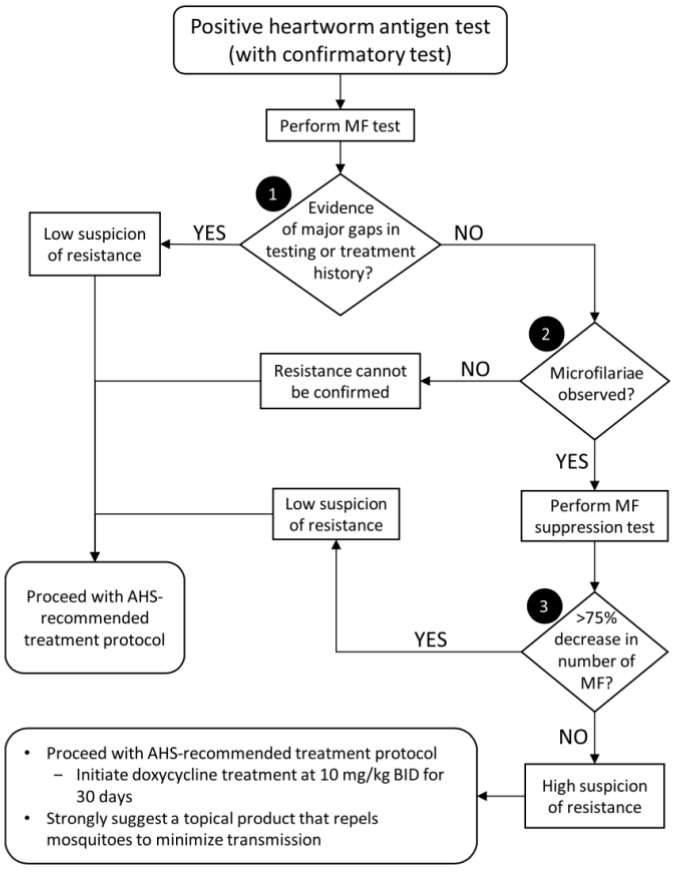

Figure 6 Decision tree ML resistance[66]

[65] Moorhead, Andrew R.; Evans, Christopher C. et al (2017) A diagnostic algorithm for evaluating cases of potential macrocyclic lactone-resistant heartworm. Parasite & Vectors 10: 1-5 (3)

[66] Moorhead, Andrew R.; Evans, Christopher C. et al (2017) A diagnostic algorithm for evaluating cases of potential macrocyclic lactone-resistant heartworm. Parasite & Vectors 10: 1-5 (3)

6.4 Efficacy of different macrocyclic lactones against resistant strains

In point 4.3, it becomes clear that ivermectin, moxidectin, and milbemycin oxime are the most used ML in heartworm treatment. Different studies evaluated the efficacy of MLs on resistant strains.

Moxidectin is becoming increasingly popular against ML-sensitive strains because of its good pharmacokinetic features and high lipophilicity. If the monthly oral dose is increased from 3 μg/kg to 24 μg/kg and given more often, it shows efficacy against ML-resistant strains. Most studies on ML against ML-resistant strains were conducted with moxidectin alone. The new research focused on the use of the commercial chewable tablet Simparica Trio®, consisting of an increased moxidectin dosage (24-48 μg/kg), together with the nematicide pyrantel and the ectoparasiticide sarolaner. Simparica Trio® was compared with Heartgard Plus®, consisting of ivermectin (6.2-11.8 μg/kg) and pyrantel, and Interceptor Plus®, which contains milbemycin oxime (0.5-1.0 μg/kg) and praziquantel against the resistant strain ZoeLA.[67]

Altogether, 24 beagles were used, and six dogs were assigned to each treatment group (T01-04). On the day of the *D. immitis* L3 inoculation, the beagles were six months old and tested negative for adult *D. immitis* antigen and blood microfilariae. Furthermore, they have not obtained any ML during the last 90 days. The L3 inoculation occurred 30 days before the first treatment and was injected subcutaneously in the inguinal region. The ZoeLA strain was collected from a naturally infected dog. The treatment was performed on days 0,30,60,90,120 and 150. There was a placebo group (T01) given empty hydroxypropyl methylcellulose capsules. Simparica Trio® was administered to group two (T02), Heartgard Plus® to group three (T03), and Interceptor Plus® to group four (T04). During and before the treatment, blood was collected on multiple days to evaluate blood microfilaria and *D. immitis* antigen testing. On days -63, -35, and 60, blood was collected to discover heartworm infection before the experiment, and on the other day, it was collected to reveal infection due

[67] Myers, Jamie A. E.; Holzmer, Susan et al (2022) Preventive efficacy of six monthly oral doses of Simparica Trio®, Heartgard Plus®, and Interceptor Plus® against a macrocyclic lactone- resistant strain (ZoeLA) of heartworm (Dirofilaria immitis) in dogs. Parasites & Vectors 15: 1-9 (2)

to the L3 inoculation. During day 241, all dogs were euthanized to perform a necropsy and investigate the adult *D. immitis* worm recovery.[68]

Day 180 revealed a negative *D. immitis* antigen and microfilariae test in group T02 treated with Simparica Trio® for five out of six dogs. The only dog left was positive for the antigen but negative for the microfilariae test. Sometime later, on days 210 and 236, two dogs tested positive for *D. immitis* antigen and negative for microfilariae. The remaining dogs were negative for antigens as well as microfilariae. All dogs of group three, treated with Heartgard Plus®, tested positive for antigens and microfilariae on days 180, 210, and 236. There was only one exception on day 236 for one dog that tested negative for microfilariae. For the Interceptor Plus® treated dogs, every animal was positive for *D. immitis* antigens and microfilariae on day 180. On days 210 and 236, five dogs were positive for microfilariae. The remaining dog was negative for microfilariae but positive for antigens.[69]

The adult heartworm ZoeLA strain counts revealed that the geometric mean for the Simparica Trio® treatment group was far lower than for Heartgard Plus® and Interceptor Plus®. The heartworms of Simparica Trio® treated dogs had a geometric mean of 1.0, whereas Heartgard Plus® had a mean of 32.5 and Interceptor Plus® 22.8 worms. This corresponds to the same value as the placebo group, with a geometric mean of 35.5 worms (**Figure 7**).[70]

[68] Myers, Jamie A. E.; Holzmer, Susan et al (2022) Preventive efficacy of six monthly oral doses of Simparica Trio®, Heartgard Plus®, and Interceptor Plus® against a macrocyclic lactone- resistant strain (ZoeLA) of heartworm (Dirofilaria immitis) in dogs. Parasites & Vectors 15: 1-9 (2-5)

[69] Myers, Jamie A. E.; Holzmer, Susan et al (2022) Preventive efficacy of six monthly oral doses of Simparica Trio®, Heartgard Plus®, and Interceptor Plus® against a macrocyclic lactone- resistant strain (ZoeLA) of heartworm (Dirofilaria immitis) in dogs. Parasites & Vectors 15: 1-9 (5-8)

[70] Myers, Jamie A. E.; Holzmer, Susan et al (2022) Preventive efficacy of six monthly oral doses of Simparica Trio®, Heartgard Plus®, and Interceptor Plus® against a macrocyclic lactone- resistant strain (ZoeLA) of heartworm (Dirofilaria immitis) in dogs. Parasites & Vectors 15: 1-9 (5-8)

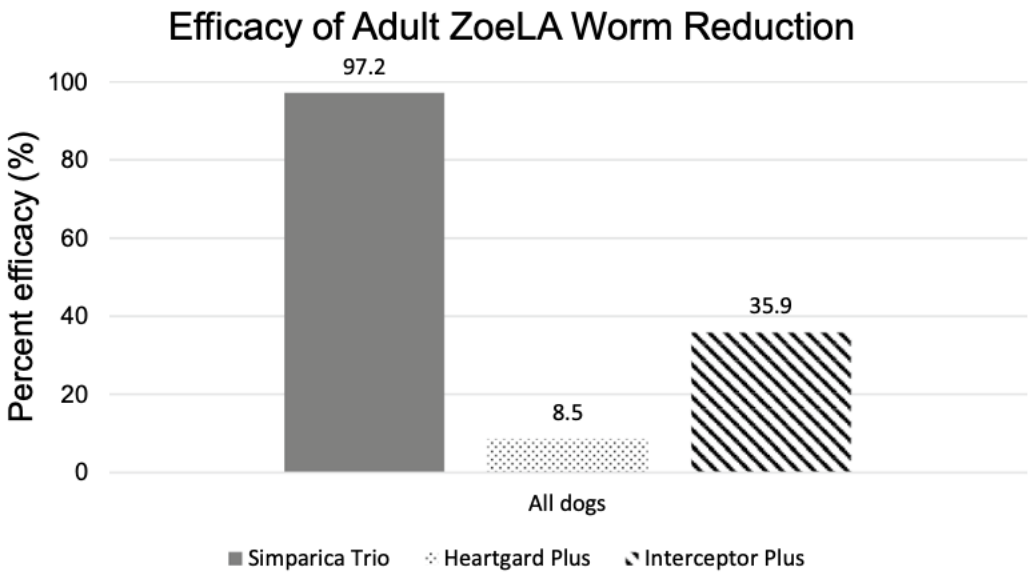

Figure 7 Efficacy[71]

Regarding the result of the study, moxidectin is the ML that has the greatest effect on heartworms. Oral moxidectin, as in Simparica Trio®, with a minimum dosage of 24 μg/kg, has been demonstrated to be outstanding against ML-susceptible strains of *D. immitis*. It was more successful if administered for six months in a row than Heartgard Plus® with ivermectin and Interceptor Plus® with milbemycin oxime.[72]

Another study compared ProHeart 12® (T02), containing 0.5 mg/kg moxidectin, and administered as a single subcutaneous injection, Heartgard Plus® (T03) and Interceptor Plus® (T04), against the ML resistant JYD-34 of *D. immitis*. The original conditions were the same as in the previous study, with six dogs in each treatment group, and the dogs tested negative for *D. immitis* antigen and blood microfilariae. Furthermore, as mentioned before, they have not received any ML treatment during the last 90 days. Like before, the *D. immitis* strain JYD-34 was also collected from a naturally infected dog. Because of two different inoculation days, the study was subdivided into two subgroups, study 1 and study 2.[73]

During study 1, all dogs were inoculated with L3 30 days before the beginning of the treatment. In study 2, the administration took place 165 days after the administration of

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71 Myers, Jamie A. E.; Holzmer, Susan et al (2022) Preventive efficacy of six monthly oral doses of Simparica Trio®, Heartgard Plus®, and Interceptor Plus® against a macrocyclic lactone- resistant strain (ZoeLA) of heartworm (Dirofilaria immitis) in dogs. Parasites & Vectors 15: 1-9 (6)

72 Myers, Jamie A. E.; Holzmer, Susan et al (2022) Preventive efficacy of six monthly oral doses of Simparica Trio®, Heartgard Plus®, and Interceptor Plus® against a macrocyclic lactone- resistant strain (ZoeLA) of heartworm (Dirofilaria immitis) in dogs. Parasites & Vectors 15: 1-9 (6-9)

73 McTier, Tom L; Holzmer Susan et al. (2021) 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. Parasite Vectors 14: 1-10 (1-4)

ProHeart 12® and 15 days after the sixth dose of Heartgard Plus® or Interceptor Plus®. Dogs in the placebo group (T01) received a subcutaneous injection with saline on day 0. The dogs in T02 were treated with an injection at day 0, and animals in T03 and T04 received the chewable treatment on days 0, 30, 40, 90, 120, 150, 180, 210, 240, 270, 300, and 330. For study 1, on days -36 and 61, blood was collected for an antigen and microfilariae test to evaluate whether the heartworm infection existed before the start of the experiment. Blood collection on days 121, 151, and 178 occurred to detect an infection because of the L3 inoculation. In study 2, the same was done on days -11 and 63 and on days 331 and 360. All dogs from study 1 were euthanized on day 185, and dogs from study 2 on day 360.[74]

On days 151 and 178, from study 1, dogs in the placebo group and all dogs treated with Interceptor Plus® tested positive for antigens. Looking at the results of Heartgard Plus®, five out of six dogs were positive for antigens on days 151 and 178. The remaining dog was antigen-negative on day 151. As for the microfilariae, all dogs in the placebo, Heartgard Plus®, and Interceptor Plus® group tested positive. Dogs treated with ProHeart 12® were the only ones negative for antigens and microfilariae on both days.[75]

The animals from study 2, in the placebo, Heartgard Plus®, and Interceptor Plus® group, tested positive for antigens on days 331 and 360. Furthermore, on day 331, all dogs were amicrofilaremic, and five from T01, T03, and T04 were positive for microfilariae on day 360. Dogs treated with ProHeart 12® were all negative for antigens on day 331, and only one was positive on day 331. In the case of microfilariae, all dogs were negative on both days.[76]

ProHeart 12® was 100% successful against the JYD-35 strain if the treatment started 30 days after L3 inoculation. This is proven because no heartworms were found during the necropsy. All dogs treated with Heartgard Plus®, and Interceptor Plus® had a geometric mean of 26.8 and 25.5 worms. This means that the success rate of Heartgard Plus® was only 10.5%, and that of Interceptor Plus® was 14.6%.[77]

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74 McTier, Tom L; Holzmer Susan et al. (2021) 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. Parasite Vectors 14: 1-10 (4-5)

75 McTier, Tom L; Holzmer Susan et al. (2021) 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. Parasite Vectors 14: 1-10 (5)

76 McTier, Tom L; Holzmer Susan et al. (2021) 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. Parasite Vectors 14: 1-10 (5)

77 McTier, Tom L; Holzmer Susan et al. (2021) 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. Parasite Vectors 14: 1-10 (5-6)

The study revealed that injectable moxidectin was the best for the ML-resistant JYD-24 strain. It has an efficiency of 100% if administered in dogs inoculated with the heartworm larvae 30 days before the treatment starts. For dogs that were inoculated 165 days after the treatment, the effectiveness is over 98%.[78]

[78] McTier, Tom L; Holzmer Susan et al. (2021) 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. Parasite Vectors 14: 1-10 (4-10)

7 Conclusion

In conclusion, the literature review highlighted the importance of MLs in heartworm disease in dogs. Unrecognized or untreated heartworm disease can lead to severe clinical symptoms in dogs, which makes prophylaxis against *D. immitis* even more fundamental. If regular prophylaxis is not provided, testing of the animals is advised to achieve optimal effectiveness and diagnose the disease at an early stage.

MLs are especially effective against L3 and L4 infection, and the preventive dose has a slow-kill effect on adult heartworms. Ivermectin, milbemycin oxime, moxidectin, and selamectin are mostly MLs used against *D. immitis*. Side effects in the case of ML use in dogs are rare but can appear in the case of ML overdose and intoxication or in Mdr1 dogs, which are very sensitive to MLs.

Due to the common use of MLs, ML-resistant *D. immitis* strains have developed, and a loss of effectiveness has been reported. Particularly in America, ML-resistant strains are increasingly frequent. A significant challenge is the diagnosis of ML-resistant strains in dogs. At the moment, there are no clinically acceptable tests that allow for uncomplicated and fast detection. Several studies demonstrate that from the MLs ivermectin, milbemycin oxime, and moxidectin. Moxidectin is the most efficient against ML-resistant strains like ZoeLA and JYD-34.

Overall, MLs are the primary class of drugs used to prevent the disease of *D. immitis* in dogs. Educating the pet owners that yearly prophylaxis is necessary to prohibit an infection ensures the optimal dosage. The correct dosage is significant not only to avoid an ML intoxication but also to prevent the development of more ML-resistant strains. In case of an underdose or irregular administration, the surviving heartworms can develop a resistance to the MLs, which are difficult to treat.

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11 Thesis progress report

UNIVERSITY OF VETERINARY MEDICINE, BUDAPEST
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INTERNATIONAL STUDY PROGRAMS
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Thesis progress report for veterinary students

Name of student: Leonie Koenes

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Department: Internal Medicine

Thesis title: Literature review about the use of macrocyclic lactones in heartworm disease

Consultation – 1st semester

	Timing			Topic / Remarks of the supervisor	Signature of the supervisor
	year	month	day		
1.	2023	05	12	Thesis topic discussion	
2.	2023	05	17	Discussion of the general content and the most important points	
3.	2023	05	22	Exchange of literatur materials	
4.	2023	06	19	Thesis announcement form	
5.	2023	06	21	Update on the current thesis status	

Grade achieved at the end of the first semester: ....5....

Consultation – 2nd semester

	Timing			Topic / Remarks of the supervisor	Signature of the supervisor
	year	month	day		
1.	2023	12	11	Submission of the table of contents	
2.	2024	01	24	Feedback of the table of contents	
3.	2024	07	03	Meeting about the thesis progress	
4.	2024	11	4	Correction of the current thesis status	

5.	2024	11	22	correction and final revisions of the thesis	

Grade achieved at the end of the second semester: ....5....

The thesis meets the requirements of the Study and Examination Rules of the University and the Guide to Thesis Writing.

I accept the thesis and found suitable to defence,

.....
signature of the supervisor

Signature of the student: ....K. Kovács.....

Signature of the secretary of the department: Gábriné Kolonics Mária

Date of handing the thesis in...2024. 11. 28.....