

Heartworm

General information



In general, filarial nematodes are characterised by their location in the deeper tissue of the host's body and their dependence upon blood-feeding arthropod vectors for transmission. In companion animals, the most important filarial nematode with zoonotic potential is *Dirofilaria immitis*,

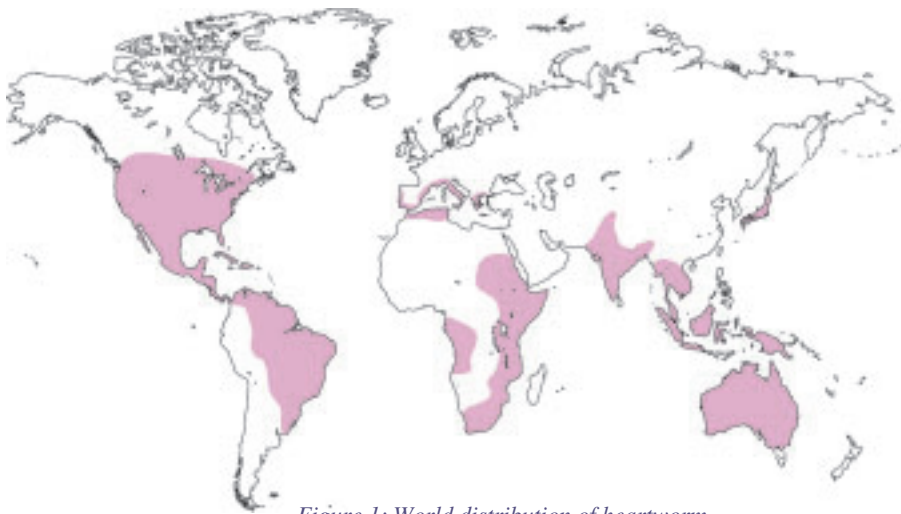


Figure 1: World distribution of heartworm

the causative agent of canine cardiovascular dirofilariasis, better known as canine heartworm disease. The closely related *Dirofilaria repens* is found in the subcutis of dogs and has zoonotic potential as well (Figure 1).

Table 1: Taxonomy of <i>Dirofilaria immitis</i>	
Phylum:	Nemathelminthes
Classis:	Nematodea (Nematoda)
Ordo:	Filarioidea
Family:	Filariidae
Sub-Family:	Filariidae
Genus:	Dirofilaria
Species:	<i>Dirofilaria immitis</i> (Leidy 1856) <i>Dirofilaria repens</i> (Raillet u. Henry 1911)

The parasite

Worms of the genus *Dirofilaria* are elongated thin nematodes, with a round anterior part. *Dirofilaria immitis* in particular is a long, slender nematode of 20 to 30 cm in length. The cuticle does not show any striations. The mouth part features a rudimentary buccal capsule without lips and six small cephalic papillae. The muscular and glandular region of the oesophagus is without distinction. The caudal region in males has a typical loose, rolled spiral, which is common in filarial nematodes, and the end is more conical than in the female worm. Male worms measure about 12 - 20 cm in length and 0.7 - 0.9 mm in width; females are larger at 25 - 31 cm in length and 1.0 - 1.3 mm in width. Dogs and wild carnivores (e.g. coy-



Figure 2: *Aedes aegypti*

otes, wolves, foxes) are the definitive hosts for *D. immitis*, however, the parasite has been reported from a wide range of mammals such as cats, wild felids, ferrets and also even sea lions.

Table 2: *Dirofilaria immitis* and other filarial nematodes reported for dogs and cats

Species and size	Definitive hosts (Accidental hosts)	Distribution	Vector species	Location of Adults	Location of Microfilaria	Pathology
<i>Dirofilaria immitis</i>	Dog, wild canids, felids (Man)	USA, Canada, Latin America, Mediterranean Region, Africa, Southern Asia, Australia, Japan	Mosquitoes (Culicidae)	Pulmonary Arteries, Heart	Blood	Cardiovascular Dirofilariasis
<i>Dirofilaria repens</i>	Dog, Cat, Fox, wild canids and felids (Man)	Southern Europe, Africa, Asia	Mosquitoes (Culicidae)	Subcutis	Blood, Skin	Occasional skin reactions
<i>Dipetalonema reconditum</i>	Dog, Hyena, Jackal	Southern Europe, Africa, North- and South America, Caribbean, Asia (Japan, India) Australia	Fleas	Subcutis, Body Cavities, Abdominal Organs	Blood	non pathogenic
<i>Dipetalonema grassi</i>	Dog	Southern Europe, Africa, South America	Ticks (Ixodidae)	Connective Tissue (Subcutis, Musculature)	Skin	non pathogenic
<i>Dipetalonema dracunculoides</i>	Dog, Fox, Hyena	Southern Europe, Africa, Asia	Ticks (Ixodidae), Keds (Hippoboscidae)	Peritoneal Cavity	Blood	non pathogenic
<i>Brugia malayi</i>	Man, Monkey, Cat, Dog etc.	South and East Asia	Mosquitoes (Culicidae)	Lymphatic Vessels	Blood	Lymphangitis, Lymphadenitis
<i>Brugia pahangi</i>	Dog, Cat, Monkey etc.	South-East Asia	Mosquitoes (Culicidae)	Lymphatic Vessels	Blood	Lymphangitis, Lymphadenitis

In addition to *D. immitis* in southern and south-eastern Europe, Africa and Asia, *D. repens* is frequently found. Furthermore, *Dipetalonema spp.*, another filarial nematode, is present in the dog and other canids. While *D. repens* and *Dipetalonema* adults reside in the subcutis, microfilaria are released into the blood stream. This may interfere in diagnosis between the pathogenic *D.immitis* and the other filarial nematodes, which are non- or only mildly pathogenic (Table 1).

Development of the Parasite

The adult nematodes of *D. immitis* are found primarily in the pulmonary arteries, the right ventricle and atrium of the heart, and occasionally, in heavy worm burdens, in the caudal Vena cava. Additional identification of adult worms in locations such as the peritoneal cavity, internal organs such as the kidneys, central nervous system and others has been reported. These

findings do not play any role in the life cycle of the parasite, however, they may have clinical implications. The females are ovoviviparous and liberate larvae, called microfilaria, which can be found in the peripheral blood. Microfilaria may survive in the blood of infected hosts for up to 2.5 years.

Circulating microfilaria are taken up by numerous mosquito species, such as *Culex*, *Aedes* and *Anopheles*, during blood feeding (Figure 2). Microfilaria have a circadian as well as seasonal rhythm, with high numbers around dusk and dawn – the main feeding times of mosquitoes. The mosquitoes are capable of harboring development to the infective larval stage. The microfilaria migrate within 24 hours from the digestive tract of the mosquito to the Malpighian organs. After about 6 - 7 days, they moult to the larval stage two (L2) and at around 9 - 15 days they transform to the L3 stage. After another 3 - 4 days, the L3 are fully grown and capable of infesting the next host during blood feeding by the

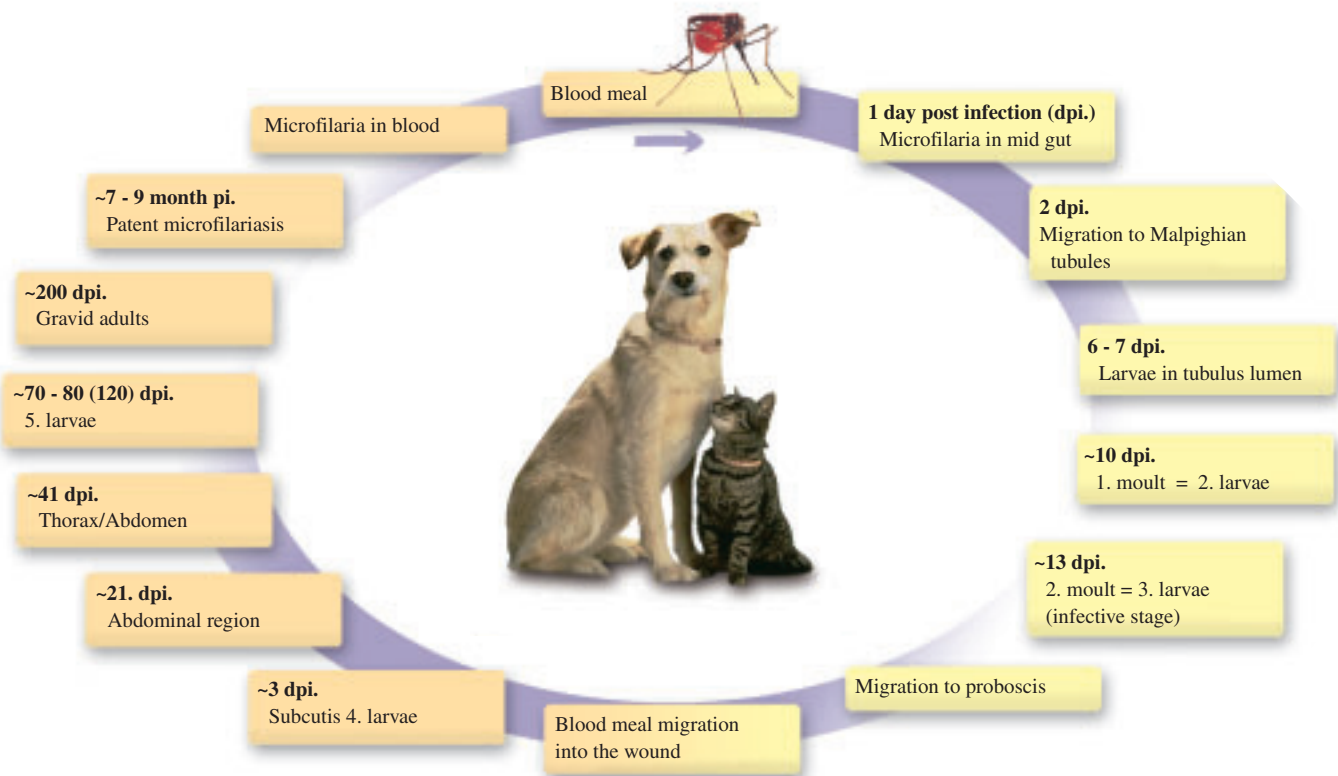


Figure 3: Life cycle of *Dirofilaria immitis* in dogs and cats

mosquito. Stimulated by positive thermotaxis, L3 larvae migrate during blood feeding of the mosquito to the tip of the mosquito's mouth parts (labium) and leave the mosquito. The L3 then enters the host via the skin lesion produced by the feeding mosquito. L3 migrate within the subcutis of the host animal where they moult 3 - 12 days post infection (dpi) to the L4 stage. The final moult to the adult stage takes place approximately 70 days after infection, during the migration towards the heart. The pre-adult stages reach the heart about 70 - 120 dpi. On about 170 to 190 dpi, the adults are fully mature and the females carry microfilaria, thus the pre-patent period in dogs is calculated to be about 7 - 9 months post infection (Figure 3). Microfilaria increase in numbers and remain constant for more than 5 years, and disappear approximately 7 - 9 years later. The normal life span of adult worms is calculated to be about 5 years.

A special situation occurs when the dog is infected with either only males or females, without microfilaria present. This situation is referred to as occult infection and describes sterile, unisex, ectopic or pre-patent infections.

Canine Heartworm (*Dirofilaria immitis*)

Pathogenesis and Clinical Appearance

Heartworm disease is regarded as a serious and potentially life threatening disease in dogs and cats caused by the adult stages of *Dirofilaria immitis* (Figure 4).

The initial pathological findings are lesions of the endothelial cells of the pulmonary arteries. Swelling of the endothelium and adhesion of neutrophils and

platelets causes lesions and allows albumin, plasma and blood cells to escape perivascularly. These endothelial changes are followed by intimal swelling and further changes in the internal arterial surface. Proliferation of the endothelium is reported to be directly related to the duration of infection as well as the worm burden.

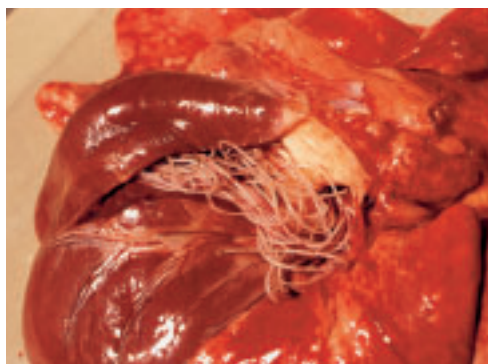


Figure 4: Adult *D. immitis* worms in the right heart of a dog

Following the vascular changes, the leaking of proteins and fluid into the lung tissue causes edema of the lung parenchyma. These changes may be seen 3 to 5 months post infection. Due to the reduced function of the pulmonary arteries, a "Cor pulmonare" with hypertrophy and dilatation of the right ventricle occurs, indicating right-sided congestive heart failure. Spontaneous death of worms can produce thromboembolism and severe inflammation.

The development of clinical signs in dogs occurs with a high level of individual variation and is dependent upon duration of the infection, the worm burden, and the activity of the dog. In general, exercise leads to more severe clinical signs of disease. Coughing is the first sign of infection, together with exercise intolerance and abnormal lung sounds. With the presence of congestive heart failure, ascites, dyspnea, tricuspid cardiac murmur and hemoglobinuria are typical clinical signs.

Diagnosis

For diagnosis of *Dirofilaria immitis* infection, parasitological, immunological and molecular techniques are available.

The parasitological test methods are filter-tests, the hematocrit-method, the Modified Knott's test or a direct blood test. All of these tests are useful in identifying a patent infection by the detection of circulating microfilaria. For the 'Modified Knott's test', 1 ml of EDTA blood mixed with 9 ml of a 2 % formalin solution is centrifuged. The sediment will contain the microfilaria. Through using methylene-blue dye, lysis of the erythrocytes occurs and the stained microfilaria are better visible. For fast detection in the clinic, one may use fresh drops of EDTA-blood mixed with physiological saline solution on a slide for direct microscopic examination. For the 'filter test', the microfilaria will be examined after blood has been pressed through a fine filter-membrane. With the hematocrit method, microfilaria will be present just above the buffy coat of leukocytes. All of these methods are highly specific, while the sensitivity may vary. Microfilaria are not present in dogs with pre-patent infections or in those with occult infections.

Immunological methods are frequently used today in detecting circulating antibodies or antigens. Tests detecting antibodies produced against an infection with *Dirofilaria* were the first commercially available test kits. Today, antigen tests are in place both for in-clinic use, as well as through diagnostic laboratories. Test sensitivity today will allow for detection of an infection with one or more adult female worms. Sensitivity decreases in infections with pre-patent worms of less than 5 months of age.

Clinical Efficacy of Advocate

During the process of product development, dose determination and dose confirmation studies with a range of endo- and ectoparasites in both dogs and cats were carried out. In these studies, the dose rate and concentration of imidacloprid was fixed to 10 mg/kg body weight and so was the dose volume. For moxidectin, it was necessary to adjust the dosage by adding different concentrations of moxidectin to the formulation. In general, moxidectin is reported to be highly effective against heartworm larvae even at very low dosages. In these dose finding studies, followed by dose determination studies, it was concluded that pre-adult

Table 3: Differential diagnosis of the microfilaria within blood of infected dogs

Typical features of microfilaria	<i>Dirofilaria immitis</i>	<i>Dirofilaria repens</i>	<i>Dipetalonema reconditum</i>	<i>Dipetalonema dracunculoides</i>
Length (m)	205 - 283	260 - 308	213 - 240	246 - 258
Mean length (m)	270	270	--	252
Width (m)	5.0 - 7.0	6.0 - 8.0	4.0 - 5.0	4.2 - 6.5
Front end	conical	Blunt	Blunt	Conical
Caudal end	straight	Hook-like bend	Hook-like bend	straight
Molecular Techniques	*PCR available	*PCR available	---	---

*PCR = Polymerase chain reaction

D. immitis were not the dose limiting parasites. Rather, gastrointestinal nematodes required a higher dosage than that necessary for heartworm control (see Chapter 7, Gastrointestinal Nematodes, for further information). All following heartworm studies were conducted using the 2.5% moxidectin concentration.

Efficacy studies

A. Efficacy beyond 30 day old larvae:

A study was conducted to determine the efficacy of Advocate on 45 day old heartworm larvae¹. In this study, dogs were experimentally infected with 50 L3 larvae removed from *Aedes aegypti* mosquitoes. At day 45 post infection, dogs were either treated with Advocate or the two actives imidacloprid or moxidectin alone. All dogs were examined for the presence of adult heartworms at day 164 post infection (day 119 post treatment). No adult heartworms were recovered from the Advocate treated dogs, nor the moxidectin treated dogs. However, a mean number of 37.6 adult *D. immitis* were found in the imidacloprid only treated dogs.

Conclusion:

This study clearly confirmed the efficacy of Advocate for heartworm prevention, even when administered to dogs with 45 day old heartworm larvae. Thus, the recommended monthly interval of Advocate application gives an extra margin of assurance in cases of non-compliance with label instructions.

B. Efficacy and water exposure/shampooing

A prerequisite of topically applied products for heartworm prevention is the evaluation of the effect of water exposure, as well as shampooing, on efficacy. Studies were carried out to determine the efficacy post-treatment with bathing/

shampooing, as well as for simulated swimming/rain exposure². In one of these studies, dogs were infected with 50 L3 *D. immitis* larvae on day 0 and treated with Advocate 30 dpi. The 40 dogs in this study were divided into 5 groups of 8 dogs each. Dogs in one group were exposed to water 1 hour post treatment and those in another group on days 1, 7, 14, 21 and 28 post treatment. To determine the effect of shampooing, the dogs were either washed 4 hours post treatment or 1 day post treatment. One group of dogs was left untreated, while receiving water exposure on a weekly basis. All dogs were examined for the presence of adult heartworms at day 153 post infection (119 post treatment). No adult heartworms were recovered from any of the Advocate treated dogs following water exposure or shampooing. In contrast, a mean of 21.7 *D. immitis* worms were recovered from the untreated control dogs.

In a second study with a similar study design as the previous study, dogs were either treated with Advocate or placebo (12 dogs each). Shampooing was performed 90 minutes post treatment. All dogs were examined for adult heartworms 146 dpi (113 dpt.). No adult *D. immitis* were recovered from the Advocate treated dogs, however, a total of 426 adult worms were recovered from the placebo treated dogs (mean of 35.5 worms/dog).

Conclusion:

From both of these studies, one can conclude that Advocate was highly effective in prevention of heartworm disease. The efficacy was not diminished when dogs were shampooed and rinsed with water 4 hours post-treatment. In addition, water exposure, as may occur with swimming or heavy rainfall, 60 minutes post treatment or with multiple exposure beginning one day post treatment did not reduce the efficacy.

C. Effects on adult heartworm in dogs

The effect of Advocate on dogs with patent heartworm infection was studied using dogs with naturally acquired heartworm infections³. Sixteen adult dogs, with even numbers of both sexes, were confirmed heartworm positive using the modified Knott's test for circulating microfilaria. Dogs included into the study had a minimum of 1,000 microfilaria/ml blood. In addition, antigen of *D. immitis* was detected using a commercially available antigen test (DiroCHEK®, Synbiotics). Dogs were divided by random into a treatment group, receiving 5 x the recommended dosage of Advocate, or a placebo group, treated with 5 x the placebo vehicle only. Treatment with five times (5x) the recommended dosage resulted in 54 - 136 mg imidacloprid/kg b.w. and 13.5 - 34 mg moxidectin/kg b.w. Dogs were monitored clinically, haematologically and by clinical pathology. On final examination 33 dpt., there was no significant difference in adult worm counts between the 5x treatment group and the placebo dogs. No unusual haematology or clinical pathology findings were recorded from dogs in either group. The microfilaria counts were found to be significantly lower in the treatment than in the control group.

Conclusion:

It can be concluded from this study that a single topical administration of up to 5 times the recommended dosage of Advocate in heartworm positive dogs demonstrated no safety concerns. Although the product may be safely administered to dogs infected with adult heartworms, it has no therapeutic effect against adult *D. immitis*. It is therefore recommended that dogs in areas endemic for heartworm should be tested for existing adult heartworm infection before treatment.

Feline Heartworm (Dirofilaria immitis)

Pathogenesis and Clinical Appearance

Cats are a suitable host for *D. immitis*, however they are somewhat less susceptible to heartworm infections. If cats get infected with heartworms, they typically harbour low numbers of worms, usually less than 6 adult worms, often of the same sex. Higher worm burdens have been reported, however, this is a rare finding. Worms that make it to maturity are smaller in size than those of patent infected dogs and the life span appears to be only 2 to 3 years, in comparison to 7 years and more in dogs. The pre-patent period is reported to be 8 months and, thus, 2 months longer than in dogs. Adult worms may be present, however microfilaremia is unusual. In general, it appears that cats are capable of eliminating worm infections spontaneously. All of these factors contribute to the fact that the prevalence of heartworm infections in cats in endemic areas is underestimated. The reasons for this include unclear or transient clinical signs, limitations in diagnostics, or, in some cases, sudden death without confirmation of the presence of heartworms.

In contrast to dogs, small numbers of heartworms are potentially life-threatening to cats. The affected organ is the lung and the pulmonary arteries. Clinical signs develop when the immature adults enter the pulmonary arteries, which may result in acute inflammation of the vessels or the lung parenchyma. Macrophage numbers increase dramatically. This first phase of infection is reported to coincide with either severe clinical signs or even death. In cats that overcome this stage, heartworms develop to the adult stage. The following phase of patent infection may be either without clinical signs or

that of a chronic unspecific infection with coughing, asthma-like signs, dyspnea, lethargy and the like. A second life-threatening stage is the spontaneous dying and degeneration of adult worms. This may cause inflammation of the lungs and thromboembolism.

Diagnosis

Diagnosis of heartworm infection in cats is more complicated than that in dogs. Circulating microfilaria are absent, clinical signs are often unspecific and radiography might miss the typically low worm burden. Additionally, diagnostic test kits developed for detection of canine heartworm infection are based on circulating female antigens, and these antigens may be absent in cats. Today, specific antibody test kits are available for diagnosis in felines. In general, it may be advisable to implement several different diagnostic tools to either determine heartworm infection in a cat or exclude heartworm infection in a cat with unspecific clinical signs.

Efficacy of Advocate

Heartworm prevention in cats

For evaluation of heartworm prevention, Advocate was studied in experimen-

tally infected cats. In a critical efficacy study, Advocate was tested in comparison to imidacloprid, moxidectin and a placebo treated control group⁴. Ten cats per group were infected with 100 L3 *D. immitis* on study Day-30 and treated on Day 0. In a follow up efficacy confirmation study, 20 cats were infected with 100 L3 larvae each 30 days prior to treatment. Cats were treated using Advocate on day 0 at moxidectin dosages between 1.04 to 1.69 mg/kg b.w. Heartworm antigen tests were performed using the DiroCHEK[®] test prior to infection on Day-35 and at day 90 post infection. All cats tested antigen negative on both days. On final examination day 140 pt. (170 dpi), all cats in the Advocate treated group were heartworm negative, while 31 adult heartworms were recovered from 8 of the 10 infected cats (average 3.25 adult HW/cat).

Conclusion:

The results of these studies demonstrated that 10% imidacloprid (Advantage) applied topically to cats had no effect on *D. immitis* larvae. Imidacloprid combined with moxidectin in the Advocate formulation, does not interfere with the efficacy of moxidectin against *D. immitis* larvae. Moxidectin either alone or combined with imidacloprid into the Advocate spot on formulation was 100% efficacious in preventing the development of *D. immitis* to the adult stage in cats.

References

- 1.- 3. Bayer internal research studies, data on file.
4. Arther RG, Bowman DD, McCall JW, Hansen O, Young DR (2003) Feline Advantage Heart (Imidacloprid and Moxidectin) topical solution as monthly treatment for prevention of heartworm infection (*Dirofilaria immitis*) and control of fleas (*Ctenocephalides felis*) on cats. *Parasitol. Res.* S90, 136-138.

Further Reading

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- Boreham, PFL, Atwell RB (eds.) (1988) *Dirofilariasis* CRC Press, Boca Raton, FL. USA.

Web-links:

- <http://www.heartwormsociety.org/heart.htm>
- <http://www.ovcnet.uoguelph.ca/PathoBio/Heartworm/HEARTWORM.html>
- <http://www.avma.org/care4pets/default.htm>
- <http://www.aavp.org/>
- <http://www.ava.com.au/>
- <http://www.parasite.org.au>