



ESDA

European Society of Dirofilariosis and Angiostrongylosis

GUIDELINES FOR CLINICAL MANAGEMENT OF CANINE HEARTWORM DISEASE

Prepared for and approved by the Executive Board of ESDA

Infection of dogs with *Dirofilaria immitis* has been diagnosed in many European countries and is spreading. These guidelines, developed by the European Society for *Dirofilaria* and *Angiostrongylus*, are based on the latest

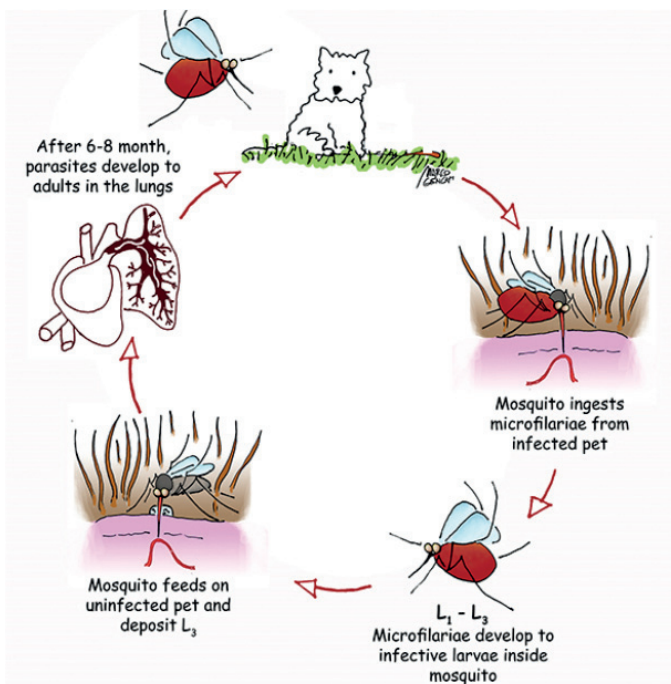
information and include up-to-date recommendations for the prevention, diagnosis, and clinical management of heartworm disease (HWD).

Life cycle of *Dirofilaria immitis*

Dogs with HWD harbor adult parasites (females approximately 25-31 cms, males 12-20 cm) in the pulmonary arteries. Microfilariae circulate in blood and are taken up by mosquitoes. Approximately 15 days later, larvae become infective L3 that are introduced into a new host. Following several months of tissue migration, parasites arrive in the pulmonary artery and begin to release microfilariae.

European Prevalence

The maps below show the European distribution of the parasite (Fig. 1a) and the most recently reported mean prevalence rates of *D. immitis* in different European countries in dogs not receiving prophylaxis (Fig. 1b). The movement of infected dogs, the presence of competent mosquito vectors and climate changes that allow the development and survival of mosquitoes for longer periods of the year all contribute to the spread of infec-



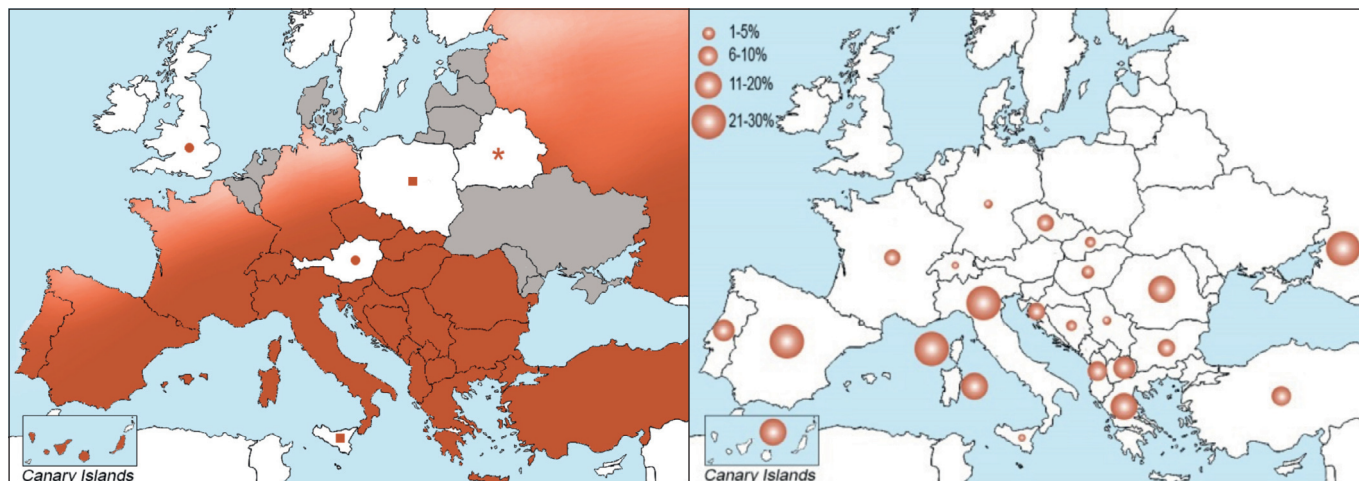


Figure 1a - *Dirofilaria immitis* in Europe.

● Imported cases only; ■ sporadic; ★ DNA of the parasite found in mosquitos; grey areas: no data.

Figure 1b - Mean prevalence rates in Europe.



Figure 2 - Numerous adults of *Dirofilaria immitis* removed from an infected dog (females on the left side, males on the right).

tion and disease. This is why it is so important to administer preventives during the transmission season. In some areas of Europe, this means year-round treatment (see section “Prevention”).

Clinical presentation of Heartworm Disease (HWD)

HWD is a chronic disease involving primarily pulmonary arteries and lungs. The heart is involved only in the last stage of infection when pulmonary hypertension leads to *Cor pulmonale* and right congestive heart failure. At times acute clinical signs can be observed in the late stage of the disease (pulmonary thromboembolism, Caval Syndrome*).

Many dogs may show no symptoms for months/years unless there is an exaggerated worm burden and/or they undergo strenuous exercise. The clinical presentations common in dogs with HWD include:

- Coughing, dyspnea
- Syncope following pulmonary hypertension
- Peritoneal effusion following right heart congestive failure
- *Caval Syndrome, which is due to a sudden rise in pulmonary pressure and the subsequent displacement

of worms from the pulmonary artery into the right cardiac chambers. Dyspnea, loud heart murmur (right side of the thorax) and hemoglobinuria are pivotal clinical signs in this syndrome.

Thoracic Radiographs (both latero-lateral and dorso-ventral views)

Radiographies are useful for evaluating the severity of pulmonary lesions. Changes include:

- perivascular inflammation
- enlargement and tortuosity of pulmonary arteries
- right heart enlargement
- pleural effusion following right heart congestive failure

It is crucial to remember that alterations of the pulmonary arteries precede cardiac involvement. Enlargement of the right cardiac chambers is not due to HW unless accompanied by changes and enlargement of the pulmonary arteries. **The severity of observed lesions is not always well correlated with worm burden.** Indeed, middle-aged dogs, particularly if sedentary, may harbor a large worm burden without severe radiographic changes. On the other hand, old dogs may present signs of previous thromboembolism with few or no worms still present.

For a step-by-step illustration of how to interpretate thoracic radiographs, consult the ESDA website: www.esda.vet

B mode, M mode and Doppler Echocardiography

This allows evaluation of pulmonary hypertension, right heart damage heartworm presence and estimating worm burden.

For a step-by-step illustration of how to interpretate Echocardiography consult the ESDA website: www.esda.vet

Clinical Pathology

Clinical-pathological abnormalities during heartworm infection are aspecific, may not always be present and are commonly related to an inflammatory state. Marked changes in hematology and biochemistry are often seen only in the late stage of the disease, or when acute changes take place (*Caval Syndrome*). Commonly seen

are leukocytosis, non-regenerative normocytic normochromic anemia, absolute eosinophilia and neutrophilia. More rarely thrombocytopenia can also be found, especially when disseminated intravascular coagulopathy (DIC) is present.

Changes in clinical biochemistry may include azotemia, a rise in liver enzymes and hyperbilirubinemia, whereas urinalysis may indicate proteinuria.

Acute phase protein (APPs) concentrations can change significantly during infection with *D. immitis*. C-reactive protein (CRP) in particular increases and this is more marked in dogs with clinical signs and vascular disease. Therefore, the use of CRP for staging the disease and monitoring recovery after treatment is recommended.

The levels of several biomarkers such as cardiac troponin I (cTnI), myoglobin, creatine kinase MB (CK-MB) increase in dogs with high parasite burden, due to myocardial injury. Dogs with more severe clinical signs of disease have higher levels of cTnI, myoglobin and CK-MB and are likely at greater risk for adverse events associated with adulticidal therapy.

D dimer levels increase after pulmonary thromboembolism (PTE) caused by natural or drug-related death of adult worms. Evaluation of d-dimer levels may therefore allow assessment of the risk for PTE, as well as aid in monitoring therapeutic success and disease progression or regression.

Diagnosis

Diagnosis of HWD in dogs (Table 1) is based the detection of microfilariae (e.g. blood smear, Knott test, filtration) and/or detection of circulating heartworm antigens (Ag). These must be taken together, and interpreted along with, results from clinical examination, laboratory tests, and results of thoracic imaging.

Modified Knott test for microfilariae - Technique

- Mix 1.0 mL of EDTA venous blood with 9.0 mL of 2% formalin in a conical centrifuge tube (a 2% formalin solution can be prepared by diluting a standard 4% or 10% formalin solution for histology with distilled or tap water).
- Invert the tube gently 4 times to mix the solution.



Figure 3 - Heartworms observed into the right cardiac chambers on necropsy.

- Centrifuge for 3 minutes at 1500 g.
- Pour off the supernatant and add 1-2 drops of 1% methylene blue and mix.
- Place a drop of the sample on a glass slide and cover with a coverslip.
- Examine the slide under the microscope at 10x to assess the presence of mf, and at 40x to observe the morphological features.
- For maximum sensitivity, the whole sediment should be analyzed.

The species identification of mf in areas where other filariae reside can be carried out by examining the morphological features. When there is doubt, specialized laboratories can also perform histochemical or molecular identification.

For a step-by-step illustration of how to perform tests for the presence and identification of microfilariae, consult the ESDA website: www.esda.net

Antigen testing

The detection of circulating antigens of *D. immitis* is a highly sensitive diagnostic technique (Table 2). Antigen testing can be used for screening asymptomatic dogs and

TABLE 1 - SPECIFIC LABORATORY TESTS FOR HWD

Tests	Description	Advantages	Disadvantages	Comments
Fresh blood smear	Vital and mobile <i>mf</i> in fresh blood	Rapid, inexpensive	Very low sensitivity, frequently false negative, poor species differentiation	Instructive for the client as it allows visualization of live parasites
Microhematocrit capillary	Concentration of the <i>mf</i> in the buffy coat of a microhematocrit capillary tube	Rapid; often carried out for routine diagnostics	Lower sensitivity than Knott test/Filtration; requires a specific centrifuge	Smear and stain of the buffy coat is required for speciation of the <i>mf</i>
Knott test/ Filtration test	Concentration and staining of circulating <i>mf</i>	High sensitivity, test of choice for the differentiation of species, inexpensive	Sensitivity and specificity are operator dependent, use of formalin is mildly problematic	Requires formalin
Detection of circulating Ag	Detection of circulating Ag with ELISA and immunochromatographic commercial kits	Very specific and sensitive, identification of "occult" infection	Costly, not diagnostic for other filarial nematodes that may be present. No detection of males or immature females	Infections with a low parasite burden may give a false negative result

mf microfilariae; *adult worms present but no circulating microfilariae.

TABLE 2 - HOW TO INTERPRET TEST RESULTS

Knott test	Ag test	Interpretation	Comment
●	●	Negative	False negatives for both tests include young parasites or young animals. Repeat after 7 months
●	●	Positive	Rx and cardiac US should be carried out for staging (see "Treatment")
●	●	Positive	False negatives for Ag tests include low mature female worm burden; Rx and cardiac US should be carried out for staging (see "Treatment")
●	●	Positive	False negatives for mf include unisex infections and the use of macrocyclic lactones (MLs); Rx and cardiac US should be carried out for staging (see "Treatment") If history and/or clinical picture are poorly compatible with HW, rule out <i>A. vasorum</i> , <i>S. lupi</i> . If prevalence in the area is very low, consider repeating using a different Ag test and/or both Knott and Ag test 3 months later

to confirm clinical suspicion of HWD. It is strongly recommended to test for both microfilariae and antigens, for maximum diagnostic performance.

Currently available antigen tests detect a protein produced mainly in the reproductive tract of adult female parasites. The most common commercially available antigen tests include enzyme-linked immunosorbent assay (ELISA) and immunochromatographic tests.

Circulating antigens are detectable only when the female heartworms develop to the adult stage, hence antigen testing should not be carried out earlier than 7 months after exposure to infection.

Antigen tests are highly sensitive (>95%) and specific (97-99%). To obtain reliable and reproducible results, antigen tests must be performed in strict compliance with the manufacturer's instructions.

Low worm burdens, with few or no adult females, can result in significantly decreased sensitivity of antigen tests (60-70% with 1-2 adult females). Furthermore, in some dogs, antigen-antibody complexes may entrap antigens making them unavailable for immunologic detection, resulting in false negative tests.

Given the high specificity of antigen testing, a positive result in areas with a high prevalence should generally be accepted as a true positive. Nevertheless, false positive results can occur and a positive result in low prevalence or non-endemic areas should be evaluated carefully. Cross reactions (false positive result) with *Angiostrongylus vasorum* and *Spirocerca lupi* infection using some heartworm antigen tests have been reported.

Several precautions are necessary in order to carry out laboratory testing that will give reliable results:

- carry out all tests only in dogs ≥ 7 months of age to

avoid false negatives

- use blood samples taken in the evening, which have more microfilariae than during the day
- perform Ag tests according to the manufacturer's instructions
- the routine heating of blood samples for Ag testing is **not recommended**.

Treatment

Adulticide treatment

Dogs with HWD harbor different stages of the parasite (microfilariae, migrating larvae, adult worms) and each stage is more or less susceptible to treatment. The bacterial endosymbiont *Wolbachia* is present in all stages and including antibiotics (doxycycline, minocycline) in the adulticide protocol has been shown to be beneficial. Staging the disease is necessary in order to choose the best treatment protocol for dogs with HWD. The death of adult worms is necessarily associated with thromboembolism and it is necessary to evaluate post-treatment response and determine the prognosis. Staging is based on the severity of pulmonary hypertension and worm burden.

Staging

History, careful clinical examination, thoracic radiographs and echocardiography are necessary for a correct staging of the patient.

Table 3 summarizes the staging of dogs and their classification as either low or high risk for thrombo-embolic complications.

TABLE 3 - STAGING OF DOGS WITH HWD

Class 1. Low risk of thromboembolic complications. (low worm burden and no parenchymal and/or pulmonary vascular lesions)	Class 2 High risk of thromboembolic complications. (High worm burden and/or parenchymal and/or pulmonary vascular lesions)
Dogs that have all these conditions	Dogs that have at least one of the following:
No symptoms	Symptoms related to the disease
Normal thoracic radiographs	Abnormal thoracic radiographs
Low level of circulating antigens or a negative antigen test with circulating microfilariae	High level of circulating antigens
No worms visualized by echocardiography and no evidence of pulmonary hypertension	Worms visualized by echocardiography and/or evidence of pulmonary hypertension
No concurrent diseases	Concurrent diseases
Owners agree exercise restriction	No likelihood of exercise restriction

Melarsomine dihydrochloride

Melarsomine kills adult heartworms and it is the first line drug for adulticide treatment.

Patient preparation for melarsomine dihydrochloride

It has been reported that the administration of preventive doses of a macrocyclic lactone (ML), combined with an antibiotic that is active against *Wolbachia*, may be used as preparation for adulticide treatment with melarsomine dihydrochloride. Thirty days of antibiotics (doxycycline at 10mg/kg bid) and two monthly doses of a macrocyclic lactone (ML) will eliminate migrating larvae and circulating microfilariae, while allowing time for older larvae to reach the pulmonary arteries and become susceptible to melarsomine. Furthermore, this regime will also weaken adult worms and make them less harmful to the lungs following their death. Dogs should continue indefinitely with monthly doses of a ML to avoid reinfection.

Melarsomine dihydrochloride administration

It is administered at the dose of 2.5 mg/kg body weight, via deep intramuscular injection in the lumbar muscles. The recommended treatment regime is a three-injection protocol, with the first injection being followed at least 30 days later (dogs with severe infection or post-adulticide complications can wait longer for completion of therapy) by a further two injections of the same dose 24 hours apart. This protocol kills the worms in a gradual manner, thus reducing the severity of pulmonary thromboembolism. Reported side effects, which usually disappear after 24-48 hours, include:

- mild swelling and soreness at the injection site
- reluctance to move due to pain at injection site
- depression
- panting
- anorexia and vomiting.

Supportive treatment during adulticide therapy

There are several recommendations and supportive treatment options during adulticide therapy, including:

- Exercise restriction (no running, no jumping, no hunting), starting from the day of diagnosis until at least one month after the last adulticide injection. This point is very important and it should be stressed to the owner that this will minimize problems associated with thromboembolism.
- Routine use of prednisone at 0.5 mg/kg bid the 1st week, 0.5 mg/kg sid the 2nd week, 0.5 mg/kg every other day for the 3rd and 4th weeks post adulticide treatment seems to reduce lung inflammation surrounding dead worms. It has been shown however that prednisone causes an increase in D dimer levels after adulticide therapy and could therefore increase severity of thromboembolism.
- **Non steroidal anti-inflammatory drugs including aspirin are not advised.** Due to the lack of evidence of clinical benefit and possible side effects
- Calcium heparin: 50-100 UI s.c. 3 times daily starting 1-2 weeks prior to and continuing for 4-6 weeks after adulticidal treatment
- Clopidogrel. Loading dose of 10 mg/kg p.o. on day 1, followed by a maintenance dose 2-3 mg/kg/sid. Is anecdotally useful but no clear evidence of efficacy
- Low molecular weight heparin, promising for other

kinds of thromboembolic disease in dogs is anecdotally useful but no clear evidence of efficacy

Antigen testing to confirm treatment success should be carried out at 6 months after the last adulticide injection. If the test is still positive, it should be repeated after a further 2-3 months. It is also necessary to control that microfilariae have been eliminated. If the dog has not been prepped using macrocyclic lactones and doxycycline (microfilariae usually fully eliminated in approximately 3-7 months), it is recommended to use a registered microfilaricide.

Alternative adulticide treatment with macrocyclic lactones and doxycycline

ML/doxycycline combinations have been shown to be adulticidal in both experimentally and naturally-infected dogs. Most studies have concentrated on preventive doses of ivermectin, either weekly or bi-weekly for 6 months, combined with doxycycline at 10mg/kg either sid or bid. Infected dogs usually begin to be negative for circulating antigens at about 12 months from the beginning of therapy. Treatment is well-tolerated with minimal radiological and clinical signs. The adulticide effects of moxidectin combined with doxycycline have also been shown following nine monthly doses of topical moxidectin combined with thirty days of 10 mg/kg doxycycline bid. This protocol results in the rapid elimination of microfilariae (by 21 days), thus breaking the transmission cycle of the parasite very quickly. Furthermore, most dogs become antigen negative by ten months, indicating an adulticidal effect. Long-term follow-up and clinical assessment of naturally infected dogs treated with this protocol are on-going. As with melarsomine, exercise restriction is recommended with this protocol during the whole treatment.

Minimally invasive Surgical Heartworm removal

Surgical removal of worms is the only treatment option for dogs with caval syndrome and must be performed immediately. If successful, clinical signs should disappear quickly. Fluid therapy may be necessary in critically ill, hypovolemic dogs to restore hemodynamic and renal function.

Surgery is also the option of choice for dogs with heavy worm burdens at risk for severe, post-adulticide complications. Echocardiographic visualization of the pulmonary arteries should be performed to determine that a sufficient number of worms are in accessible locations. Antigen testing is not advised following surgical heartworm removal, due to the persistence of antigenemia. Dogs can be rechecked after at least 6 months to determine (together with the clinical picture) if additional adulticide treatment is necessary.

Treatment of spontaneous or post-adulticide thromboembolism

- Cage rest (mandatory)
- Oxygen supplementation (Inspired oxygen > 40 %)

TABLE 4 - PREVENTION PROTOCOLS IN DIFFERENT CLINICAL SCENARIOS

Age	Puppies <2 months	Start on chemoprophylaxis as soon as possible according manufacturers recommendations		
	Puppies 2-7 months	Start on chemoprophylaxis	Test 7 months after the beginning of the chemoprophylaxis	If negative or positive year-round prevention If positive: adulticide treatment
	Animals older than 7 months	Test previous chemoprophylaxis efficacy - HW Ag and	If negative: Start on chemoprophylaxis and re-test after 7 months to exclude recent infections	If negative or positive year-round prevention If positive: adulticide treatment
			If positive: adulticide treatment	
	Lack of compliance	Noncompliance in chemoprophylaxis >7 months	Test before resume chemoprophylaxis Knott test	If negative: Start on chemoprophylaxis and re-test after 7 months to exclude recent infections If positive: adulticide treatment
	Noncompliance in chemoprophylaxis <7 months	Resume chemoprophylaxis	Test 7 months after the beginning of the chemoprophylaxis	If negative or positive year-round prevention If positive: adulticide treatment
Travel to endemic areas	Travel from a heartworm-free country to an endemic country for <1 month	Receive 1 monthly dose of macrocyclic lactones when return to the heartworm-free area		
	Travel from a heartworm-free country to an endemic country for >1 month	Start 1 month after the beginning of the travel and end 1 months after returning to the heartworm-free area		

- Prednisolone helps to control the clinical signs of pulmonary thromboembolism (cough, dyspnea, fever, weakness, hemoptysis), at the dosage of 1-2 mg/kg sid for 3-4 days, thereafter reducing the dosage on the basis of clinical signs
- Calcium heparin 200-300 IU 3 times a day s.c.
- Butorphanol (up to 0.4 mg / kg i.m. q 2-4 hours) or Morphine (up to 0.3 mg / kg s.c. q 4-6 hours) if severe respiratory distress is present
- Sildenafil (1-3 mg/kg) every 8-12 hours or Tadalafil (1 mg/kg every 48 hours) orally have been anecdotally reported as useful.
- Diuretics are not advised

Treatment of right heart congestive failure

If effusions are present

- Furosemide 1 mg/kg 2-3 times a day or Torasemide 0.1 mg/kg once a day oral route
- Spironolactone 2 mg/kg once a day
- Hydrochlorothiazide 0.5-1 mg/kg 1-2 times a day if refractory effusion
- Angiotensin-converting-enzyme inhibitors (carefully checking blood pressure and renal function)
- Digoxin 0.005-0.01 mg/kg 2 times daily (only if atrial fibrillation is present for decreasing heart rate)

TABLE 5 - MACROCYCLIC LACTONES USED FOR THE PREVENTION OF HWD

Drug	Administration	Dose	Interval	Efficacy against other ESDA parasites #
Ivermectin	Oral	> 6 mcg/kg	Monthly	<i>D. repens</i>
Milbemycin	Oral	> 0.5-0.75 mg/kg	Monthly	<i>A. vasorum</i>
Moxidectin	Injection SR	0.17 mg/kg	every 6-12 months	<i>D. repens</i>
Selamectin	Spot on	6 mg/kg	Monthly	
Moxidectin	Spot on	>2.5 mg/kg	Monthly	<i>A. vasorum, D. repens</i>

At the labelled dose, all these drugs **are safe** in dogs that **are sensitive to MLs** due to the presence of the so-called Multidrug Resistant 1 mutation (for example, Collies, Australian shepherds, etc.).

Prophylactic activity against *D. repens* or *A. vasorum*. Some drugs alone or in combination are active against other endo or ectoparasites.

- Phosphodiesterase 5 inhibitors (Sildenafil and Tadalafil) and Phosphodiesterase 3 inhibitors (Pimobendan) may not be useful due to the anatomical and physiological modification of pulmonary arteries

Prevention

HWD can be prevented (Table 4) by the administration of macrocyclic lactones (Table 5) that are able to eliminate infective larvae up to 30 days old. Thus, the monthly administration will kill all the larvae that mosquitoes have introduced in the previous 30 days. The slow-release injectable formulation available in some countries maintains the larvicidal effect for the 6-12 months.

The current recommendation is to carry out prevention all year round; even in winter, urban heat islands allow mosquitoes to survive. Below are some key points to

prevention in different clinical situations.

The use of topical synthetic pyrethroids (i.e. permethrin), applied monthly, has been reported as significantly decreasing the risk of mosquito bites in dogs (so-called “anti-feeding” effect). **The use of pyrethroids does not substitute the use of MLs**, but it can be combined with macrocyclic lactones to lessen the risk of infection in the case of missed administration of the ML (lack of compliance) by the owner (“double defense”).

Retesting

All dogs on correct prevention should be screened (both microfilariae and antigen testing) every other year. If failure of administration of one or more doses or lack of owner compliance is suspected, annual retesting is mandatory.



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