Diagnosing Heartworm Disease in Dogs: Why, When, and How

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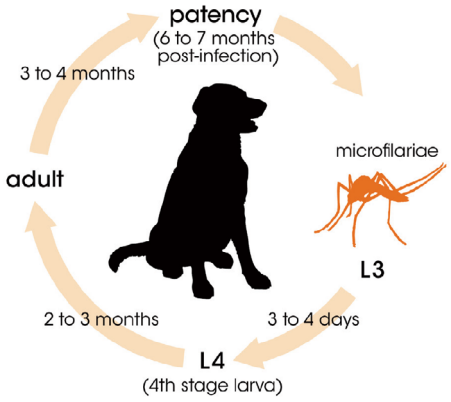
Introduction

Despite the decades long availability of effective heartworm disease preventatives, now available in many different forms (oral, injectable, and topical), heartworm prevalence in dogs has increased in the United States by 21% from 2013-2017.[1] Dogs are potentially at risk of heartworm infection, at least regionally, in all 48 contiguous states in the U.S., as well as in Hawaii, Puerto Rico, and the U.S. Virgin Islands.[2]

Because of the long lifespan of adult worms (5-7 years), clinical signs of heartworm disease may not occur for months to years after infection, once severe and irreversible pathology has occurred (pulmonary hypertension and right sided heart enlargement and failure); however, pathological changes in the pulmonary arteries begin as early as 3-5 months after infection.[3] Because clinical signs are not evident in many infections, annual screening is strongly recommended to identify and treat heartworm-positive dogs before severe disease occurs.[2] Early diagnosis and treatment can also reduce the transmission of heartworms via mosquitoes to unprotected dogs, which is essential to curbing the continued spread of this deadly infectious disease.

Heartworm disease is on the rise despite the availability of effective preventives.

Clinical signs of heartworm disease may not occur for years after infection, but pathologic changes can begin to occur within 3-5 months after initial infection.



Heartworm transmission factors:

Four factors are necessary for heartworm transmission to occur:

1. The presence of mosquitoes capable of transmitting heartworm
2. Climate conducive to the development of infective heartworm stages in mosquitoes
3. Heartworm-infected dogs/canids with circulating microfilariae (reservoirs)
4. Susceptible canid hosts

Over 60 species of mosquitoes are capable of transmitting heartworm disease in the world, and at least 25 species are present in the United States.[4] Heartworm larval development in the mosquito requires temperatures of at least 57°F, and the speed of development is directly proportional to temperature.[5] Under experimental conditions, larval development pauses in lower temperatures, and resumes after temperature increases above 57°F.[5] After an infective mosquito completes a bloodmeal, the infective L3 larval stage released in the hemolymph enter the skin and subcutaneous tissues through the mosquito bite wound. Once in the subcutaneous tissues, the L3 larvae will molt to the L4 stage within a few days. L4 larvae will then grow and migrate towards the pulmonary arteries. These two stages are the susceptible targets of heartworm disease preventives. The final molt to Immature adults, less susceptible to macrocyclic lactone preventives, occurs as early as day 50 and as late as day 70 post infection.[4]

Why test every dog, every year?

Heartworm disease is progressive and is less reversible the longer a dog is infected. Disease severity is correlated with the number of worms and duration of infection. Early diagnosis decreases the risk of developing clinical signs, subclinical damage, and enhances the opportunity for a full recovery.

The American Heartworm Society recommends heartworm screening as part of an annual wellness exam for every dog.

Because heartworm disease has a high morbidity and affects mortality, annual heartworm screening should be part of an annual wellness visit for every dog. The American Heartworm Society (AHS) recommends both annual antigen and microfilaria testing for all dogs over 7 months of age, regardless of lifestyle, geographic location, or history of prophylaxis.[2] This recommendation is based on the fact that the earliest that heartworm antigen and microfilariae can be detected is approximately 5 and 6 months post infection, respectively.[2]

Antigen detection is the most sensitive and specific diagnostic test available.

Heartworm disease prevalence has increased nationally. In addition to warmer climates and new invasive mosquito vectors, one main change in recent years is the translocation of heartworm-positive dogs to relatively lower prevalent heartworm regions in the U.S. after natural disasters and overcrowding of shelters in the southern U.S.[1,7] The introduction of these heartworm-infected dogs can lead to an increase in infected mosquitoes when they feed on infected dogs, and thus an increased risk of infection to the local canine population. One study demonstrated that 7 out of 10 mosquitoes collected in the backyard of a heartworm infected dog contained infective larvae.[8] Despite increases in heartworm disease prevalence, heartworm disease preventative compliance has remained stagnant from 2013-2016, with only 1 in 3 dogs in the United States dispensed heartworm disease preventive by their veterinarian at an average of 8 months of doses administered per year.[9]

Infection with drug-resistant isolates of heartworms is a risk, especially in dogs living in or near the lower Mississippi River Valley region. Macrocyclic-lactone resistant heartworm infections have been detected in dogs in Illinois, Tennessee, Arkansas, Louisiana, Mississippi, and Alabama.[10] The impact of the expansion of drug-resistant isolates in the U.S. after movement of dogs from these areas is yet to be determined, although there is one known case of a dog with a drug-resistant heartworm infection transported to Ontario, Canada after hurricane Katrina.[11] Every-other-year or nontesting in relatively lower prevalent areas and/or in dogs on compliant heartworm prevention should be avoided due to the increase in prevalence of heartworm infection nation-wide, known poor preventive medication compliance, and the potential for infection with drug-resistant isolates.

Testing for heartworm infection

Antigen testing

Antigen detection is the most sensitive and specific diagnostic test available, and it is recommended by the AHS to be performed in tandem with microfilaria testing for all dogs.[2] The Zoetis WITNESS® and VETSCAN® Heartworm Rapid Tests are visual, qualitative point-of-care tests for the detection of *Dirofilaria immitis* antigen in canine (and feline) whole blood, plasma, or serum. In a recent study, the WITNESS and VETSCAN Heartworm Rapid Tests demonstrated a sensitivity of 99% and 98.5%, respectively, and a specificity of 94% for both tests.[12]

Despite the high accuracy of heartworm antigen tests, not every heartworm infected dog will have a heartworm positive test. In one study, 90.9% of dogs harboring 2 worms tested positive on an antigen test; however, in the case of dogs with 3-5 worms or 1 worm, antigen was detected in 75% and 55.6%, respectively.[18] Antigen may never or only sporadically be detected in dogs with a very low female worm burden or all male worm infection. Additionally, antigenemia may be suppressed until about 9 months post-infection in infected dogs receiving heartworm prophylaxis.[13] The timing of antigen testing is

critical to generate an accurate result. Table 1 is a summary of the recommended schedule of antigen and microfilaria testing in different clinical scenarios.[2]

Table 1. Schedule of heartworm testing for different clinical scenarios[2]

Clinical Scenario	Initial Test	Follow up Test 1	Follow up Test 2
< 7 months of age	Do not test; Start HWP	1 year of age*	1 year from initial test and annually thereafter
≥ 7 months of age **No or unknown history of HWP**	Test now & start HWP	6 months from initial test	1 year from initial test and annually thereafter
≥ 7 months of age **Switching to different HWP**	Test now & start HWP	6 months from initial test	1 year from initial test and annually thereafter
Missed HWP dose	Restart HWP now; Test 6 months after missed dose	6 months from initial test	1 year from initial test and annually thereafter

HWP = Heartworm preventative; Test = antigen and microfilaria testing in tandem

***1 year of age simplifies pet owner reminder and coincides with 1 year core vaccine booster series; good practice is to recheck 6 months from initial administration.**

Microfilaria testing

Before heartworm antigen testing became available, microfilaria testing was the only diagnostic test method veterinarians had available to diagnose heartworm infection. The disadvantage of this test is that the absence of microfilaria does not rule out heartworm disease. Between 20% to 47.7% of dogs with adult heartworm infection may not be microfilaremic, even in high prevalence areas.[14]

The modified Knott's test is the most sensitive method of microfilaria detection because of its concentration technique of 1 mL of blood. Because 2% formalin, conical tubes, and a centrifuge are needed to perform the test, most clinics do not perform this testing in-clinic; however, it is available at most reference laboratories. The modified Knott's test is also used during a microfilaria suppression test when determining the likelihood of a drug-resistant heartworm infection.[15]

A positive direct smear can confirm a positive in-clinic heartworm antigen test. A direct smear or wet mount can be performed in-clinic to microscopically evaluate the presence of microfilariae and confirm a positive in-clinic heartworm antigen test. However, the main disadvantage of this test is that the small volume of blood being examined can limit the identification of microfilariae. In one study, a direct blood smear detected microfilariae in 80.9% of microfilaremic samples confirmed by modified Knott's testing.[14] Direct smears detected microfilariae in all samples that contained more than 50 microfilariae per mL, but only detected 44.3% of samples that contained less than 50 microfilariae per mL. Microfilariae of *Acanthocheilonema reconditum,* a non-pathogenic parasite transmitted by fleas to dogs, can also be detected on a direct smear, and its similarities to *D. immitis* may result in a false positive interpretation. This is why it is important to confirm a microfilaria-positive dog with a heartworm antigen test.

The American Heartworm Society also recommends microfilaria testing in conjunction with antigen testing because some dogs will be heartworm microfilaria-positive and antigen negative due to immune complexes. In two separate studies evaluating naturally infected dog populations, microfilaria-positive and antigen-negative tests occurred in 0.2% and 0.3% of cases.[2,16]

Heat Pre-Treatment[2]

All dogs with heartworm disease will have some degree of antigen-antibody complexes, resulting in some antigen not being available to bind to detecting antibodies on heartworm antigen tests. However, some infected dogs will have all or most of the circulating antigen bound by antibodies in immune complexes, leading to false negative antigen test results. These dogs may or may not be microfilaria positive, and they may have low to high numbers of worms. These factors explain why the intensity of the color of the test result cannot be correlated with the number of adult worms present.[17] For example, in one study evaluating heat pre-treatment on banked serum samples

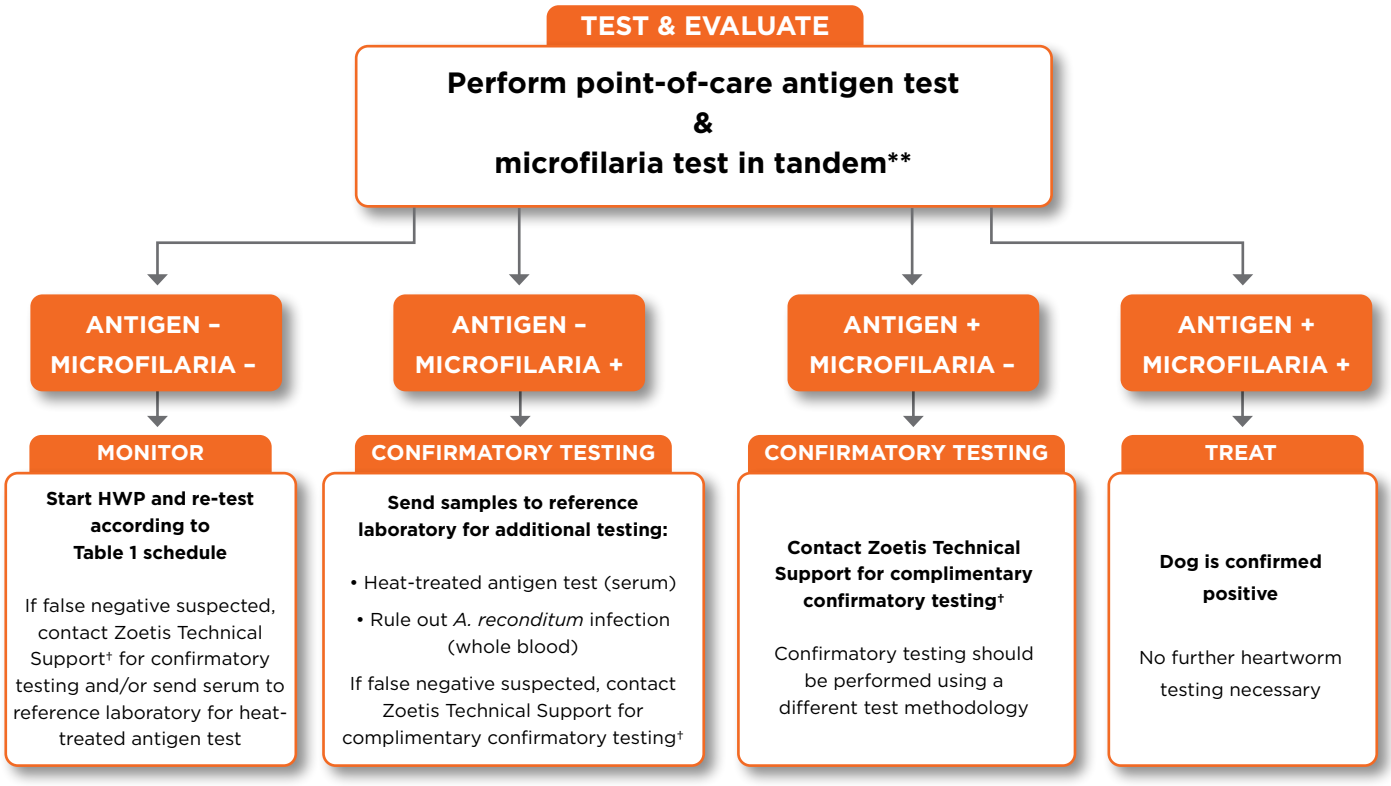
Paired microfilaria testing with antigen testing is the current AHS screening recommendation.

from necropsied dogs with known mature adult worm numbers, dogs that converted from heartworm antigen negative to positive after heat pre-treatment had a range of 1-40 worms present.[18] High heat and centrifugation of the serum sample, prior to performing the heartworm test, releases blocked antigen by denaturing antibodies bound to antigen (in complexes). This method is available through reference laboratories and should be considered when a negative heartworm antigen test is questioned based on the history and clinical picture of the patient. ***The AHS does not currently recommend routine heating of blood samples to separate antigen-antibody immune complexes as a screening test, nor for point-of-care antigen screening tests.*** Heat treatment is contrary to the label instructions and USDA testing methods used to approve antigen detection tests, such as the VETSCAN and WITNESS Heartworm Rapid Tests. However, a recent study showed that heat treatment increased the sensitivity of detection of mature heartworm infections from 90.7% to 98.4% with a decrease of specificity from 97.8% to 96.1%.[18] Although heat treatment may release blocked antigen that would otherwise cause false negative test results, further studies are needed to accurately interpret conversion of no antigen detected to antigen positive results after heat treatment.

Ancillary diagnostics

In addition to antigen and microfilaria testing, a complete blood count, chemistry profile, urinalysis and thoracic radiographs +/- echocardiography are indicated if clinical signs and/or heartworm infection are suspected.[2] Figure 1 shows a diagnostic algorithm that can be used when testing dogs for heartworm infection.

Figure 1. Interpreting canine heartworm test results*



*Ancillary diagnostic testing (thoracic radiographs ± echocardiography) should be used in conjunction with heartworm diagnostic tests when heartworm disease is suspected regardless of diagnostic test results.

**Complimentary confirmatory testing is only offered for VETSCAN and WITNESS tests

†Free confirmatory testing available for VETSCAN or WITNESS Heartworm Rapid Test through Zoetis Technical Support

HWP = heartworm preventative

How VETSCAN and WITNESS Heartworm Rapid Tests compare with other point-of-care heartworm antigen tests[12]

A 2018 peer-reviewed study published in *Veterinary Parasitology* journal evaluated five patient-side *D. immitis* antigen detection tests and one microplate ELISA test (Zoetis DiroCHEK) that served as the reference lab standard.[12] This study used archived canine serum divided into five subclasses of female worm confirmed by necropsy: 0, 1-5, 6-20, 21-40, >40. Each test was run once following the manufacturer's instructions and personnel were blinded to the *D. immitis* status of each dog.

The overall sensitivity and specificity of the patient-side kits, including the VETSCAN Heartworm Rapid Test and WITNESS Heartworm test were high: ≥98.5% and 94%, respectively (see Table 2 below for individual test sensitivity and specificity). The agreement between tests for all five subclasses was between all 99%-100%, and agreement with cardiopulmonary necropsy between 98%-99.6%. The sensitivity and specificity of the Zoetis DiroCHEK test (typically used in reference laboratories) compared to necropsy results was 99% and 96%, respectively. Agreement between DiroCHEK and all patient-side tests was between 97% and 100%.

Table 2. Sensitivity and specificity of point-of-care diagnostic compared to necropsy tests[12]

	Zoetis VETSCAN® Heartworm Rapid Test	Zoetis WITNESS® Heartworm Rapid Test	IDEXX SNAP® 4DX Plus Test	Heska Solo Step® Canine Heartworm Antigen Test	Anigen Rapid One Step
Sensitivity (95% CI)	98.5%	99%	97.5%	98%	99.5%
Specificity (95% CI)	94%	94%	94%	94%	94%

The VETSCAN and WITNESS Heartworm Rapid Tests are both highly sensitive and specific for detecting canine heartworm disease.

It is recommended to confirm all positive antigen tests prior to beginning adulticide therapy due to the possibility of false positive test results.

All point-of-care tests are considered screening tests, in which sensitivity should theoretically be higher than specificity, which was demonstrated in this study. The specificity for all tests in this study was 94%, demonstrating that false negative results can occur in any point-of-care test currently available for veterinary use. Because these are screening tests, all positive test results require additional confirmatory testing, as recommended by the AHS. If a test result on the VETSCAN or WITNESS Heartworm Rapid Test is unexpected or questionable based on the history and clinical picture, the test should be confirmed. Zoetis Technical Support can help troubleshoot, and if necessary, confirm an unexpected result using a different methodology at no charge to Zoetis customers. The intensity of the color of the test result cannot be accurately correlated with the number of worms present (a faint positive does not correlate with a low number of worms or "light" infection). Concentration tests for microfilariae, heat treatment, thoracic radiography, or ultrasonography may also aid in interpretation of antigen test results.

Summary

The prevalence of canine heartworm disease is increasing nationwide, resulting in increased risk of canine infection.[1,2] According to the American Heartworm Society, all dogs should receive year-round heartworm disease prevention.[2] However, due to compliance concerns and because clinical signs are typically not present in early infections, annual screening is recommended for all dogs in order to detect and treat heartworm infection before severe disease occurs.[2]

Canine heartworm disease is diagnosed based on clinical findings in conjunction with a point-of-care antigen test, such as the VETSCAN and WITNESS Heartworm Rapid Tests and microfilaria testing. False negative test results may occur due to early infections, low worm numbers, all male infections, blocked antigen in immune complexes, and/or failing to perform the test in accordance with manufacturer's guidelines.

Heat-treatment of samples for point-of-care heartworm antigen testing is not

recommended for screening by the AHS 2018 guidelines. However, heat treatment performed at a commercial lab is a good tool to confirm a negative antigen test that is questionable based on history and/or clinical signs.[4]

Because false positive test results can occur, these results should be confirmed with microfilaria testing and/or a different antigen test as well.[22] According to a university study, the sensitivity and specificity was statistically equivalent among all currently available point-of-care heartworm antigen tests.[12]

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