

PARASITOLOGY

FAQs and Answers About Heartworm Diagnosis

Lindsay Starkey, DVM, PhD, DACVM (Parasitology), Oklahoma State University College of Veterinary Medicine, Stillwater, Oklahoma

Tom Nelson, DVM, VCA Animal Medical Center of NE Alabama, Anniston, Alabama

The American Heartworm Society (AHS) recommends that routine heartworm screening in dogs include both an antigen test and a microfilaria test to guide treatment decisions. However, interpreting the results of these tests is not always straightforward. This article answers some of the most common questions the AHS receives about heartworm screening and diagnosis in dogs and cats.



On annual screening, a canine patient tested antigen positive, but the microfilaria test result was negative. Should I assume the patient is heartworm positive and initiate adulticide treatment?

Microfilaria testing has been recommended by the AHS

as a component of annual screening in dogs since 2016. Determining microfilaria status is important for a variety of reasons. If the dog is antigen positive, the presence of microfilariae confirms the heartworm infection and identifies the infected dog as a potential heartworm reservoir; therefore, the patient should be administered a macrocyclic lactone that is effective against microfilariae as part of the treatment phase preceding and during administration of melarsomine. A list of these products is available at heartwormsociety.org/preventives.

However, it is not uncommon for infected dogs to test antigen positive and microfilaria negative. One reason for this can be that the dog has received intermittent doses of heartworm preventive since becoming infected;

Abstract

Accurate diagnosis is an essential component of heartworm management in dogs and cats. Routine heartworm screening tests play an important role in diagnosis, as well as equip clinicians with information to assess heartworm incidence and risk in their practice areas. Because test timing and techniques can affect the accuracy of heartworm diagnosis, the American Heartworm Society often receives questions from general practitioners about heartworm testing. This article answers some of the most frequently asked questions about heartworm screening and diagnosis.



Take-Home Points

- The American Heartworm Society (AHS) recommends that routine heartworm screening in dogs include both an antigen test and a microfilaria test to guide treatment decisions. However, it is not uncommon for these tests to yield unexpected or conflicting results.
- Immune complex formation can lead to false-negative results in both dogs and cats. When a clinician suspects heartworm infection based on clinical signs but antigen is not detected, heat or acid treatment can be used to dissociate antigen-antibody complexes.
- Up to 12 times more feline heartworm infections may be diagnosed using immune-complex dissociation testing methods.
- Test timing is a crucial element of heartworm treatment follow-up.
- In a 2024 update to the AHS Feline Heartworm Guidelines, the AHS began recommending routine heartworm screening in cats. This screening includes a Heska antibody test and an antigen test with a heat-treated serum sample.

depending on the preventive, this may result in amicrofilaremia at the time of testing. Another reason might be the use of an insensitive microfilaria test. While a clinician can assume the dog is likely infected based on the positive antigen test result, a second antigen test (using a different type than the initial test) should be administered to confirm the heartworm diagnosis; alternatively, a more sensitive microfilaria test could be performed.

Once infection is confirmed, a heartworm treatment protocol that includes administration of a macrocyclic lactone, 4 weeks of doxycycline, 3 doses of melarsomine, and other adjunct medications should be initiated.

Q I recently saw a dog in my hospital that was exhibiting mild clinical signs of heartworm disease. The microfilaria test result was positive, but the antigen test result was negative. How should these results be interpreted?

Such results are less common than antigen-positive and microfilaria-negative results, but several important scenarios can lead to this situation. The most concerning is the presence of immune complexes. In recent years, studies have confirmed that immune complexes formed when heartworm antigen is bound by circulating antibodies can block the detection of antigen in an infected dog.^{1,2} This can occur in dogs exhibiting clinical signs—such as the patient described—but also in asymptomatic patients.

Presence of less common filariid species in circulation, errors in running the antigen test (including mishandling of the sample and using inadequate or diluted sample), and inherent defects in the antigen test itself are also possibilities.

A logical next step is to repeat the antigen test using a serum sample that has been heat treated (acid treatment is another possibility, but this option is not yet widely available). Heat treatment, which is available from most reference and state laboratories, can dissociate the immune complex and free the heartworm antigen for detection.

Q I recently finished treating a heartworm-positive dog using the 3-injection protocol recommended by the AHS. When should the patient be retested, and what if they continue to test positive?

Postadulthood testing is an important component of heartworm treatment and includes follow-up microfilaria and antigen testing.

Microfilaria testing has been recommended by the AHS as a component of annual screening in dogs since 2016.

Microfilaria Testing

The AHS heartworm treatment protocol recommends that dogs treated with melarsomine undergo follow-up microfilaria testing with a quantitative test 30 days after the last injection of melarsomine. This screening should be conducted regardless of whether the patient was positive or negative for microfilariae prior to treatment. If microfilariae were present at the initial diagnosis, retesting should be completed to ensure they have been successfully eliminated following elimination of adult worms. If there were no microfilariae at the initial diagnosis, it is possible that the adult worms present could have mated in the 2-month interim between diagnosis and initiation of melarsomine injections; thus, retesting ensures that the animal is not a reservoir. The latter scenario is rare but possible.

Finding microfilariae at follow-up is not necessarily a cause for concern, provided the dog was treated with the full AHS protocol, which entails (1) administration of a heartworm preventive on day 0 and compliant redosing thereafter; (2) administration of doxycycline at 10 mg/kg twice daily for 4 weeks, starting on day 0; and (3) 3 doses of melarsomine, given on days 60, 90, and 91. However, if less than the full dosage of either doxycycline or melarsomine was given, treatment efficacy could be reduced.

If the full dosages were administered and the dog remains microfilaria positive, the possibility of macrocyclic lactone resistance should be considered. To investigate this possibility, the clinician should perform or request a quantitative microfilaria test that includes the actual number of microfilariae in the sample, then administer topical moxidectin (the only FDA-approved microfilaricide). A week later, repeat the microfilaria test and count the microfilariae once again. If the number has dropped by 75%, chances are good the microfilariae being treated are not resistant.³

Adulticide treatment is up to 99% effective if the 3-injection protocol is followed.^{4,5}

Antigen Testing

Before 2018, the AHS recommended administering a follow-up antigen test 6 months after the last melarsomine injection. The recommended wait period is now 9 months, for the following reasons:

- Antigen tests today are more sensitive than they once were, and very small quantities of heartworm antigen in the blood can be detected.
- Not all adult heartworms die at once; instead, they die over a period of a month or more. Meanwhile, residual heartworm antigen in circulation is detectable for months after the worms have died. Even if adulticide treatment has eliminated 100% of the worms by the 6-month point, the presence of small amounts of remaining antigen could lead to an invalid test result. By waiting an additional 3 months to test the dog, veterinarians can feel confident in the test result and avoid unnecessary retesting and/or retreatment, which is costly as well as stressful for the pet and owner.
- By 9 months after treatment, any antigen detected can be assumed to be from worms that survived adulticide treatment or from a new infection resulting from inadequate prevention compliance. In such cases, retreatment with 30 days of doxycycline followed by 2 doses of melarsomine is recommended.

Adulticide treatment is up to 99% effective if the 3-injection protocol is followed.^{4,5} Accurate, reliable post-treatment microfilaria and antigen testing can give veterinarians and owners important peace of mind.

 **I practice in a heartworm-endemic area where I'm able to screen most of my canine patients for heartworms. Should I also be routinely screening cats? What do the latest AHS Feline Heartworm Guidelines recommend?**

There are 4 primary reasons to test cats for heartworms: (1) to help confirm a diagnosis in a cat with clinical evidence of infection, (2) to monitor the status of a cat already diagnosed with heartworms, (3) to establish a baseline prior to beginning a heartworm prevention program, and (4) to better understand the relative risk/incidence of feline heartworm in a practice area.

As of late 2024, the AHS recommends that cats be routinely screened with an *antigen test* using *heat-treated serum* as well as the *Heska antibody test*. (Note: Heartworm antigen testing that utilizes a heat- or acid-treatment step to disrupt immune complexes in plasma or serum is available through most reference



and state diagnostic laboratories.) The rationale is:

- A heartworm antigen test detects the presence of adult female worms. However, this test can produce false-negative results in the presence of antigen–antibody immune complexes, and heat pretreatment is a technique that dissociates immune complexes in blood samples. Antigen test results are also more likely to be false negative when only male worms are present.²
- Data accrued over the past several years have revealed that immune complexes occur at a concerning rate in feline heartworm infections. Heat treatment of feline samples can increase the antigen test's sensitivity by a factor of 3 to 12 times that of non-heat-treated samples and result in substantially more cats being diagnosed with heartworms.⁶⁻⁸
- A heartworm antibody test detects the presence of antibodies produced in response to a previous or existing heartworm infection at any stage, from larvae to adults. According to studies, the Heska antibody test is up to 8 times more sensitive than the Synbiotics antibody test^{9,10}; therefore, the AHS now specifically recommends use of the Heska antibody test. Veterinarians can access the Heska (an Antech company) antibody test by calling Heska at 970-493-7272 and asking for test #805514 or through the Antech test directory individually (S14544) or bundled with a heat-treated antigen test (S14546). Nevertheless, any antibody test can produce a false-negative result.

SUMMARY

Heartworm testing is an important part of diagnosis of heartworm disease; however, discordant results can and do happen. Knowing how best to navigate the different scenarios or reach out for consultation on these more difficult cases is critical for efficient and appropriate patient management.

For answers to these and other questions about heartworm testing, practitioners can consult the AHS website at heartwormsociety.org and, when needed, email questions to info@heartwormsociety.org. **TVP**

References

1. DiGangi BA, Dworkin C, Stull JW et al. Impact of heat treatment on *Dirofilaria immitis* antigen detection in shelter dogs. *Parasit Vectors*. 2017;10(suppl 2):483. doi:10.1186/s13071-017-2443-7
2. Gruntmeir JM, Long MT, Blagburn BL, Walden HS. Canine heartworm and heat treatment: an evaluation using a well based enzyme-linked immunosorbent assay (ELISA) and canine sera with confirmed

heartworm infection status. *Vet Parasitol*. 2020;283:109169. doi:10.1016/j.vetpar.2020.109169

3. Geary TG, Bourguinat C, Prichard RK. Evidence for macrocyclic lactone anthelmintic resistance in *Dirofilaria immitis*. *Top Comp Anim Med*. 2011;26(4):186-192. doi:10.1053/j.tcam.2011.09.004
4. Keister DM, Dzimiński MT, McTier TL, et al. Dose selection and confirmation of RM 340, a new filaricide for the treatment of dogs with immature and mature *Dirofilaria immitis*. Presented at: American Heartworm Society Triennial Symposium; 1992; Batavia, Illinois.
5. Keister DM, Tanner PA, Meo NJ. Immiticide: review of discovery, development and utility. Presented at: American Heartworm Society Triennial Symposium; 1995; Auburn, Alabama.
6. Little SE, Raymond MR, Thomas JE, et al. Heat treatment prior to testing allows detection of antigen of *Dirofilaria immitis* in feline serum. *Parasit Vectors*. 2014;7:1. doi:10.1186/1756-3305-7-1
7. Gruntmeir JM, Adolph CB, Thomas JE, Reichard MV, Blagburn BL, Little SE. Increased detection of *Dirofilaria immitis* antigen in cats after heat pretreatment of samples. *J Feline Med Surg*. 2017;19(10):1013-1016. doi:10.1177/1098612X16670562
8. Nelson CT, Johnson CM. Evaluation of feline heartworm disease based on gross necropsy, serology, pulmonary histopathology, and radiographic evidence in adult shelter cats in northeastern Alabama. *Parasit Vectors*. 2024;17(1):161. doi:10.1186/s13071-024-06178-9
9. Hays KM, Rodriguez JY, Little SE, et al. Heartworm prevalence in dogs versus cats: multiple diagnostic modalities provide new insights. *Vet Parasitol*. 2020;4:100027. doi:10.1016/j.vpoa.2020.100027
10. Snyder PS, Levy JK, Salute ME, et al. Performance of serologic tests used to detect heartworm infection in cats. *JAVMA*. 2000;216(5):693-700. doi:10.2460/javma.2000.216.693



Lindsay Starkey

Dr. Starkey is an associate professor at Oklahoma State University's College of Veterinary Medicine. She earned her BS degree in animal science from the University of Arkansas and completed her DVM and PhD in veterinary biomedical sciences degrees at Oklahoma State University, where her graduate research focused on several vector-borne infections of dogs. She completed her residency training through the National Center for Veterinary Parasitology at Oklahoma State University. She is involved in various research projects involving vector-borne pathogens, diagnostic parasitology, and parasite consultation and outreach. Dr. Starkey currently serves as a codirector for the National Center for Veterinary Parasitology, editor and board member for the American Heartworm Society, and vice president of the American Association of Veterinary Parasitologists.



Tom Nelson

Dr. Nelson is the hospital director of the VCA Animal Medical Center of NE Alabama in Anniston, Alabama, and has been in private practice for 45 years. He was first elected to the board of the American Heartworm Society in 2001 and has served as the society's president (2004-2007) and symposium cochair (2010-2013). Dr. Nelson is currently chair of the AHS research committee. Along with being involved in heartworm research, he is the lead author of the AHS's canine and feline guidelines. In addition, he has authored or coauthored over 30 papers and contributed 4 chapters to textbooks about heartworm disease.