Performance of serologic tests used to detect heartworm infection in cats

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Objective—To compare heartworm serum antibody (Ab) and antigen (Ag) test results, using commercial laboratories and in-house heartworm test kits, with necropsy findings in a population of shelter cats.

Design—Prospective study.

Animals—330 cats at an animal shelter.

Procedure—Between March and June 1998, 30 ml of blood was collected from the cranial and caudal venae cavae of 330 cats that were euthanatized at a local animal shelter. Results of heartworm Ab and Ag serologic tests for heartworm infection were compared with necropsy findings in this population of cats, using commercial laboratories and in-house test kits to measure serum Ab and Ag concentrations.

Results—On necropsy, adult Dirofilaria immitis were found in 19 of 330 (5.8%) cats. Combining results from serum Ab and Ag tests achieved higher sensitivities than using serum Ab and Ag test results alone (ie, maximum sensitivities of 100% vs 89.5%, respectively), whereas use of serum Ag and Ab test results achieved higher specificities compared with the use of a combination of serum Ab and Ag results (ie, maximum specificities of 99.4% vs 92.9%, respectively).

Conclusions and Clinical Relevance—On the basis of our findings, if a cat has clinical signs that suggest heartworm disease despite a negative heartworm serum Ab test result, an alternative heartworm Ab test, a heartworm Ag test, thoracic radiography, or two-dimensional echocardiography should be performed. (J Am Vet Med Assoc 2000;216:693–700)

Prevalence of heartworm infection in cats appears to be increasing. Because microfilaremia occurs transiently or never in cats infected with Dirofilaria immitis, accurate serologic tests are prerequisite to determining the infection rate in a community to assess the need for heartworm prophylaxis in cats. These tests are also essential in the diagnosis of feline heartworm disease when thoracic radiographs appear normal, are too expensive to perform, or when imaging modalities such as echocardiography or angiography are not available or are cost prohibitive. Because clinical signs can be manifested in cats harboring immature worms or in cats with as few as 1 adult worm (even 1 worm can be lethal to cats), tests that accurately detect early infections or small worm burdens are crucial in the diagnosis of feline heartworm infection and disease.

Serologic testing for heartworm infection has been used for many years in dogs. Immunofluorescent antibody tests, which detect microfilarial (cuticular) antibodies, are of limited benefit in cats because of rare microfilaraemia. The ELISA antibody (Ab) tests were once used in dogs to detect antibodies to adult D immitis but were finally abandoned because of low specificity, in part caused by cross-reactivity with other parasites. Heartworm ELISA Ab tests designed for cats are, however, available commercially and are used as screening tests for cats. In cats, gastrointestinal parasitic infections have minimal to no cross-reactivity with heartworm Ab tests. Nonetheless, the specificity of the test has been brought into question, because most of the currently available feline heartworm Ab tests detect serum Ab concentration to immature and adult heartworms. Because the Ab test results may become positive as early as 60 days after infection, and because some immature forms never complete their development into adult worms, a positive Ab test result in cats only documents exposure to migrating heartworm larvae or to adult heartworms. Antibody test results will also be positive in cats with previous heartworm infections, although the time required for results of an Ab test to become negative after a cat eliminates the infection is not known. Until recently, false-negative Ab test results were considered to be rare. However in 2 independently conducted studies, 14% of infected cats had a negative Ab test result.

The ELISA antigen (Ag) tests, which are the preferred tests to determine the heartworm status of dogs, detect a protein found primarily in the female worm’s reproductive tract. However, unlike dogs, the number of heartworms that cats usually harbor is low (1 to 4 worms) so the likelihood of infection solely with male worms is high in cats. These infections would most likely be missed by the heartworm Ag tests. Immature female worms or small numbers of female worms can also cause a negative Ag test result. For these reasons, the Ag test has not been considered to be an appropriate screening test for heartworm infection in cats; however, the rate of false-positive results is believed to be low.

Necropsy confirmation of heartworm infection has been used as the standard for determining heartworm status in dogs. Unfortunately, cats develop ectopic heartworm infections more commonly than dogs; thus, heartworm infection is easier to miss on routine examination. Precardiac infections are also missed on gross inspection. Nonetheless, gross confirmation of infection is currently the method against which perfor-
mance of various heartworm tests are judged. Objective information comparing the reliability of currently available heartworm tests for cats is not available to the practicing veterinarian. The purpose of the study presented here was to compare heartworm serum Ab and Ag test results, using commercial laboratories and in-house heartworm test kits, with necropsy findings in a population of shelter cats.

Materials and Methods

Animals and samples—Between March and June 1998, 30 ml of blood was collected from the cranial and caudal vena cavea of 330 cats that were euthanized by intraperitoneal injection of sodium pentobarbital at a local animal shelter. Blood was collected within 2 hours of euthanasia. Following blood collection, the heart and lungs were removed. Cranial and caudal vena cavea, right atrium, right ventricle, and pulmonary arteries (to approx 1 mm in diameter) were examined for heartworms. Lung specimens and worms were preserved in a 10% solution of formalin. Worm gender was established by inspection under a dissecting (10X magnification) microscope. Male worms were identified by the distinctive spiral or coiled appearance of the tail, gravidity of female worms was identified by exposing the worm’s uterine contents to examine for eggs.10,11

Blood was allowed to clot and was centrifuged, and serum was separated, stored in 1 ml aliquots, and frozen (–80°C) for later analysis or shipment. Split serum samples were shipped to commercial laboratories (HESKA Veterinary Diagnostic Laboratories [HESKA Lab]11 and Animal Diagnostics [Diagnostics Lab]12) for heartworm Ab and Ag ELISA testing. The HESKA Lab Ab results were reported as positive if the continuous numeric serum Ab concentrations were ≥ 5 U/ml.5 Because Piché reported that cats with serum Ab concentrations ≥ 20 U/ml were more likely to have positive serum Ag test results than cats with serum Ab concentrations of 5 to 19 U/ml,13 HESKA Lab Ab results were also evaluated, using a serum Ab concentration of ≥ 20 U/ml as a positive-cutpoint value. The Diagnostics Lab Ab titer results were reported as positive if the numeric titers were ≥ 1:70.

In addition, 4 in-house ELISA kits were used to detect the presence of heartworm Ab (ASSURE/FH Feline Antibody Heartworm Test Kit [ASSURE]); HESKA Solo Step FH [Solo Step]),14 and Ag (Snap Heartworm Antigen Test [SNAP]; DiroCHEK Heartworm Antigen Test Kit [DiroCHEK]) in serum samples. One senior biological scientist (MES) and 3 veterinary students performed the in-house tests. Technicians were trained by senior investigators or company representatives. All kits tested in our laboratory were labeled specifically for use in cats with the exception of the SNAP Ag test.

The SNAP Ag results were reported as negative, positive-low Ag concentration, or positive-high Ag concentration. Cats were considered to have a positive result if the scores were recorded as positive-low Ag concentration or positive-high Ag concentration. In addition to the recommended 5-minute incubation time for the Solo Step Ab test, results were also read after 10 and 20 minutes of incubation.

Personnel performing all tests were blinded to the necropsy findings and to the results of other tests. Degree of sample hemolysis was scored as none to mild (0 to 1), moderate (2), or severe (3). Degree of lipemia was scored as none (0) or mild (1). Samples were not evaluated for microfilaria.

Statistical methods—Analysis of the diagnostic performance of heartworm tests focused on evaluation of decision rules used to designate a heartworm test result as positive. Seven Ab test decision rules (Table 1) and 4 Ag test decision rules (Table 2) were evaluated. In addition, combinations of Ab tests with Ag tests for a total of 16 possible Ab/Ag pair-
ings, were evaluated using decision rules based on the occurrence of a positive test result from the Ag or Ab test considered as a single composite test (for the purposes of evaluating these Ag/Ab test pairs, HESKA Lab Ab results ≥ 5 U/ml were considered positive and Solo Step test results were read after a 20-minute incubation).

Sensitivity, specificity, and corresponding exact 95% confidence intervals were computed for each test decision rule using necropsy results (positive or negative for heartworm infection) as the standard. Simple mean of the sensitivity and specificity, which is equivalent to the area under a receiver-operating characteristic curve (AUC-ROC) when the test result and standard (ie, positive or negative necropsy finding for heartworms) are binary, was used as a measure of overall diagnostic performance for each decision rule. To compensate for the chance of making a type-I error while performing a large number of pairwise tests, the Bonferroni method was used to maintain a significance level of 0.05 across all pairwise comparisons among the individual Ab and Ag test decision rules and across all pairwise comparisons among Ag/Ab test pairs. Thus, calculated P values had to be < 0.001 for each comparison among the 11 test decision rules and < 0.0005 for each comparison among the 16 Ag/Ab test pairs to be considered significant.

Sensitivity and specificity of each test were also computed for subgroups of cats defined by blood sample hemolysis status (mild, moderate, severe), lipemia status (positive or negative), and sex (male, female). Fisher’s exact test was used to compare sensitivities or specificities between lipemia status subgroups and between hemolysis status subgroups. In comparing sensitivities among hemolysis status subgroups, blood samples from cats that had positive necropsy results with moderate or severe hemolysis (n = 11 and 3, respectively) were combined into a single subgroup.

Assuming the estimated sensitivity and specificity of each test decision rule to be fixed, Bayes’ Rule was used to compute negative- and positive-predictive values for each test given projected heartworm prevalence of 1, 5, and 10%.

Results

Necropsy findings—Adult D immitis were found in 19 of 330 cats giving a heartworm infection prevalence of 5.8% in this population of shelter cats. Mean number of worms per cat was 1.63 (median, 1 worm; range, 1 to 4 worms); 11 cats had a 1 worm. Gravid female worms were found in 3 cats. All cats with gravid female worms also had at least 1 male worm. Single sex infections were found in 14 cats (5 cats with male heartworm[s] only; 9 cats with female heartworm[s] only).

HESKA Lab Ab and Ag results—Eighty-seven of 330 (26.3%) cats had positive HESKA Lab Ab (serum Ab concentration ≥ 5 U/ml; Table 1) results. Thirty-eight of 87 (14.9%) cats also had positive HESKA Lab Ag results; 10 of these 13 cats had Ab concentrations ≥ 20 U/ml.

With a serum Ab titer ≥ 5 U/ml considered positive, HESKA Lab Ab results correctly identified 17 of 19 (89.5%) of the cats that had positive necropsy results and 241 of 311 (77.5%) of the cats that had negative necropsy results. If a positive-cutpoint serum Ab concentration of ≥ 20 U/ml had been used, 29 (8.8%) cats would have had positive results, decreasing the specificity of the Ab test to 57.9% (11/19) but increasing the sensitivity to 94.2% (293/311). Two cats that had positive necropsy results with a negative HESKA Lab Ab titer had a single worm at necropsy.

Sixteen (4.9%) cats had positive HESKA Lab Ag results (Table 2). The HESKA Lab Ag test had a sensitivity of 68.4% (13/19) and a specificity of 99.0% (308/311). Six cats that had positive necropsy results with negative HESKA Lab Ag results had a male only worm infection (4 cats) or a single female worm (2 cats).

Adult heartworms were not found on gross examination in 70 (22.5%) cats that had HESKA Lab Ab concentrations ≥ 5 U/ml and 18 (5.8%) cats that had HESKA Lab Ab concentrations ≥ 20 U/ml. Adult heartworms were not found in 3 cats that had positive HESKA Lab Ag results; 2 of these cats also had negative HESKA Lab Ab results. Two cats that had negative necropsy results (with positive HESKA Lab Ag results) had positive results on other Ag tests (DiroCHEK Ag test and Diagnostics Lab Ag test).

ASSURE Ab and DiroCHEK Ag results—Thirty-five of 330 (10.6%) cats had antibodies to D immitis on the basis of positive ASSURE Ab results (Table 1). The ASSURE Ab test had a sensitivity of 68.4% (13/19) and a specificity of 92.9% (289/311). A single worm was found at necropsy in 5 of 6 cats that had positive necropsy results with a negative ASSURE Ab result.

The DiroCHEK Ag test correctly identified 15 of 19 (78.9% sensitivity) cats that had positive necropsy results and 303 of 311 (98.1% specificity) cats that had negative necropsy results (Table 2). Three cats that had positive necropsy results had a positive DiroCHEK Ag result but negative ASSURE Ab results.

Twenty-two (7.1%) cats had positive ASSURE Ab results, but no adult heartworms were identified on necropsy. Six cats had positive DiroCHEK Ag results, but no worms were found on necropsy. One cat that had a negative necropsy result had a positive DiroCHEK Ag result. Another cat that had a negative necropsy result with a positive DiroCHEK Ag result had a positive SNAP Ag result.

Diagnostics Lab Ab and Ag results—The Diagnostics Lab Ab test was performed on serum samples from 329 cats. The test was not performed on a sample from 1 cat (heartworm negative results on necropsy) because of an insufficient quantity of serum. Forty-seven (14.3%) cats had positive Diagnostics Lab Ab results (Table 1). The Diagnostics Lab Ab results correctly identified 15 of 19 (79.0% sensitivity) cats that had positive results on necropsy and 278 of 310 (89.7% specificity) cats that had negative results on necropsy. Three of 4 cats that had positive results on necropsy with negative Diagnostics Lab Ab results had a single worm infection.

The Diagnostics Lab Ag test was performed on serum samples from 328 cats. Because of an insufficient quantity of serum, the test was not performed on serum samples from 2 cats (both cats had negative results on necropsy). Nineteen (5.8%) cats had positive Diagnostics Lab Ag results (Table 2). The Diagnostics Lab Ag test had a sensitivity of 73.7% (14/19) and a specificity of 98.4% (304/309). No cats with a positive Diagnostics Lab Ag result had a negative Diagnostics Lab Ab result.
Thirty-two cats (10.3%) had positive Diagnostics Lab Ab results, but no adult heartworms were found on necropsy. Five cats had positive Diagnostics Lab Ag results, but no worms were found on necropsy; 1 of these cats also had a positive HESKA Lab Ag result.

The Solo Step Ab results—Samples from 326 cats were tested for Ab with the Solo Step test. A sufficient quantity of serum was not available from 4 cats; none of the 4 untested cats had positive results on necropsy. Samples from 3 cats that had negative necropsy results were hemolyzed and prevented accurate reading of Solo Step Ab results, and the control line did not appear after 20 minutes of incubation in the result window for 1 additional sample. Nine of 322 (2.8%) remaining cats had a positive result using the Solo Step Ab test, according to the manufacturer’s recommendation of 5 minutes for incubation.

Six of 19 cats that had positive necropsy results had positive Solo Step Ab results after the recommended 5-minute incubation period, yielding a sensitivity of 31.6% and a false-negative rate of 1.0% (99.0% specificity; Table 1). When the test was allowed to incubate for 10 minutes, 3 additional cats that had positive necropsy results had positive Solo Step Ab results, and 14 additional cats that had negative necropsy results had positive Solo Step Ab results, increasing sensitivity to 47.4% and increasing the false-negative rate to 5.6% (94.4% specificity). When the test was allowed to incubate a total of 20 minutes, samples from 7 additional cats that had positive necropsy results had positive Solo Step Ab results, and 39 more cats that had negative necropsy results had positive Solo Step Ab results, achieving a sensitivity of 84.3% and a 14.8% false-negative rate (specificity 85.3%).

SNAP Ag results—Sixteen of 330 (4.9%) cats had positive SNAP Ag results (Table 2). The SNAP Ag test had a sensitivity of 73.7% (14/19) and a specificity of 99.4% (309/311). Five cats that had positive necropsy results with negative SNAP Ag results had a single heartworm on necropsy.

Two cats with positive SNAP Ag results did not have heartworms on gross inspection. One of these cats also had positive DiroCHEK Ag and HESKA Lab Ab results. The remaining cat had a positive DiroCHEK Ag result.

Total number of worms and the total number of female worms identified at necropsy did not appear to be associated with the magnitude of positive SNAP Ag results. Significant difference in the mean (± SD) or median number of worms was not observed between cats that had positive results for low serum Ag concentration (mean, 2 ± 1.2 worms; median, 2 worms) and those that had positive results for high serum Ag concentration (mean, 1.6 ± 0.6 worms; median, 2 worms), as indicated by the independent-sample t-test and the Wilcoxon rank sum test.

False-negative results—Although the sensitivity of serum Ab tests for heartworm disease is reported to be high, 13 of 19 cats that had positive necropsy results had at least 1 negative ELISA Ab result. Four cats that had positive necropsy results had 3 negative Ab test results, 4 cats that had positive necropsy results had 2 negative heartworm Ab test results, and 5 cats that had positive necropsy results had 1 negative heartworm Ab test result.

Eight of 19 cats that had positive necropsy results had at least 1 negative ELISA Ag result. All 4 Ag test results were negative in 2 cats; both cats had 1 male heartworm. Three of 4 Ag test results were negative in 3 cats that had positive necropsy results. Two of these cats had a single male heartworm; the other cat had a single female heartworm. One Ag test result was negative in 3 cats that had positive necropsy results; 2 of these cats had a single female worm and 1 cat had 3 female worms.

False-positive results—Depending on the Ab test performed, the number of cats with false-positive Ab results ranged from 3 to 70. The Solo Step FH test gave the fewest false-positive test results, but it also missed 13 cats that had positive necropsy results. The HESKA Lab Ab test gave the largest number of false-positive results, but missed the fewest cats that had positive necropsy results (2 cats). Twelve cats that had negative necropsy results had at least 1 positive heartworm Ag test result including 1 cat with 3 positive Ag test results and 2 cats with 2 positive Ag test results.

Comparing the diagnostic performance of individual tests—The DiroCHEK Ag test had the overall best diagnostic performance among Ag and Ab tests, achieving a sensitivity of 78.9% and a specificity of 98.1% (sensitivity-specificity mean: 88.5%). The worst performing test among all tests evaluated was the Solo Step Ab test when evaluated after a 10-minute incubation, demonstrating a sensitivity of 31.6% and a specificity of 99.1% (sensitivity-specificity mean: 63.5%). Test sensitivities ranged from 31.6% (Solo Step Ab test evaluated after a 10-minute incubation) to 89.3% (HESKA Lab Ag test, using a serum Ab concentration ≥ 5 U/ml as the positive cutpoint). These worst and best sensitivities were the only ones to differ significantly from one another. Sensitivities of other tests that were between these 2 extremes did not differ significantly from either extreme or from one another. Antigen test specificities (range: 98.1 to 99.4%) did not differ significantly from one another but were significantly greater than the 3 lowest Ab test specificities (range: 77.5 to 89.7%).

Diagnostic performance of Ab/Ag test pairs—Using the AUC-ROC as a method to measure overall diagnostic performance, the Solo Step test evaluated at 20 minutes, combined with either the HESKA Lab Ab, SNAP or DiroCHEK Ag tests, had the best performance among Ab/Ag test pairs, with sensitivities in the range of 94.7 to 100%, specificities at 83.6 to 84.9% (range of sensitivity-specificity means, 89.7 to 91.8%) and the AUC-ROC ranging from 0.90 to 0.92.

The AUC-ROC for the 16 possible combinations of Ab and Ag tests ranged from 0.83 to 0.92. Sensitivities ranged from 79.0 to 100% among all Ab/Ag test pairs, although no significant differences in sensitivity were observed between test pairs. Antibody/antigen test pairs’ specificities had a range of 76.1 to 92.9%.

When test specificity alone was examined, the test pairs involving Diagnostics Lab or ASSURE Ab tests (specificity range: 87.7 to 92.9%) had significantly higher specificities than those test pairs involving the
HESKA Lab Ab test (specificity range: 76.1 to 77.2%). Specificities of test pairs involving the Solo Step test evaluated after a 20-minute incubation (specificity range, 83.6 to 84.9%) fell between these 2 groupings, not differing significantly from either group.

In general, Ab/Ag test pairs achieved higher sensitivities than did Ab and Ag tests alone (maximum sensitivities of 100% vs 89.3%), whereas Ag and Ab tests alone achieved higher specificities compared with the Ab/Ag test pairs (maximum specificities of 99.4% vs 92.9%). This is to some extent a result of Ab tests having higher sensitivities and lower specificities than Ag tests.

Effect of hemolysis and lipemia—Samples from 91 cats including 3 cats with heartworms were considered severely hemolyzed. Samples from 151 cats including 11 cats with heartworms were considered to have moderate hemolysis. Samples from 85 cats including 5 cats that had positive necropsy results were recorded as having mild to no hemolysis. Hemolysis scores were not recorded for 3 cats. With 1 exception, the sensitivity and specificity of a test did not appear to depend on whether blood samples were hemolyzed. Specificity of the Solo Step test evaluated at 10 minutes was significantly greater \( P = 0.019 \) with moderate to severe hemolysis (214/222: 96.4%) than with mild or no hemolysis (69/78: 88.5%).

Lipemia was not observed in 209 samples, and mild lipemia was observed in 116 samples including 6 cats that had positive necropsy results. Degree of lipemia was not scored in 5 samples. Test sensitivity and specificity did not depend on lipemia status, with 1 exception. Sensitivity of the Solo Step test evaluated at 5 minutes was significantly greater \( P = 0.046 \) with mild lipemia (4/6 cats that had positive necropsy results correctly identified; 66.7%) compared with no lipemia (2/13 cats that had positive necropsy results correctly identified; 15.4%).

Discussion

Heartworm Ab test results varied widely with as few as 9 (2.8%) to as many as 86 (26.1%) cats having positive necropsy results. Degree of hemolysis after the death of worms, immature larvae or ectopic infections not detected on gross examination, or nonspecific Ab reactions. In our study, fecal examinations were not performed, so cross reactivity with other parasites was not assessed. Some cats may have had previous heartworm infections, because the length of time a cat continues to have positive Ab test results after resolving a heartworm infection is unknown. Lastly, ectopic infections were possible in our study, because visual inspection at necropsy was limited to the heart and pulmonary arteries.

Heartworm Ab tests are currently recommended as screening tests in cats, because the rate of false-negative results is reported to be low. In our study, the sensitivity of heartworm Ab tests ranged from 31.6 (Solo Step Ab test evaluated after a 5-minute incubation) to 89.5% (HESKA Lab Ab test). In 2 studies of experimentally heartworm infected cats, HESKA Lab Ab, Diagnostics Lab Ab, and the ASSURE Ab tests had sensitivities of 100% at 4 to 5 months after exposure.16 Although reported to be rare, other investigators have also reported negative Ab test results in cats naturally infected with heartworms or in cats with positive heartworm Ag test results.12,13 In the report by McCall of 215 random-source, naturally infected cats, there were 8 cats that had positive necropsy results, and 7 (87.5%) of these had positive heartworm Ab test results.1 Atkins and others reported similar positive Ab tests results (19/22; 85% cats) in naturally heartworm infected cats. The data from our laboratory also indicated that most of our cats that had positive Ab test results also had positive Ag test results. Lastly, Gecchi reported positive Ab test results using the Solo Step Ab test in 81% of naturally infected cats that did not have clinical signs and in 98% of cats with clinical signs.12

Based on a heartworm infection prevalence of 5% in our cats, the positive-predictive value for the heartworm Ab tests that we performed ranged from 73.3 to 62.7%. Although the percentage of false-positive test results was highest with the HESKA Lab Ab test (specificity of 77.5%), it identified the largest number of cats that had positive necropsy results (17/19 cats that had positive necropsy results) compared with the other Ab tests (ie, there were fewer false-negative results).

The HESKA Lab Ab test performed better than the Solo Step Ab test (when the Solo Step Ab test was used according to the manufacturer’s recommendations) even though both test for Ab to the same recombinant Ag, (ie, rHWAg1, Tables 1 and 3). When the Solo Step Ab test was allowed to incubate for 20 minutes instead of the recommended 5 minutes, the specificity of the test approached that of the HESKA Lab Ab test and the sensitivity exceeded the HESKA Lab Ab test.

Of the 2 cats that had positive necropsy results with negative HESKA Lab Ab test results, 1 cat was harboring 1 female worm. This cat had 1 other positive heartworm Ab test result and 3 positive heartworm Ag test results. The other cat with a negative HESKA Lab Ab test result had 2 other positive Ab test results and 1 positive heartworm Ag test result, even though only a male heartworm was identified on necropsy.

Positive and negative-predictive values for each test are presented (Table 3) for 3 different prevalence rates (ie, 1, 5, and 10%). Atkins and others reported a 9% heartworm infection rate in cats that have clinical signs of cardiorespiratory disease.4 Therefore, if testing was restricted to cats with cardiorespiratory signs, the positive-predictive value of all the tests would improve but the negative-predictive value of the tests would decline.
In the cats that had positive necropsy results, there was no correlation between the numbers of worms found and the concentration of Ab (when the Ab concentration was reported), which is in agreement with other reports. However, our results concur with those of Piché et al, who showed that cats with HESKA Lab Ab results of serum Ab concentrations of ≥ 20 U/ml are more likely to have positive Ag test results than cats with an Ab concentration of 5 to 19 U/ml. However, if a serum Ab concentration of 20 U/ml was relied upon as the positive-cutpoint value, almost a third of the cats that had positive necropsy results in our study would have been considered to have negative Ab test results. Watkins reported that 79% of cats with Diagnostics Lab Ab results of titers > 1:3,000 also had positive Ag titers. The highest Diagnostics Lab Ab titer measured in our study was 1:575.

Because the Ab tests detect Ab to different Ag, it is not surprising that there was some discrepancy in results. Heartworm Ab tests developed by HESKA Veterinary Diagnostic Laboratories (HESKA Lab Ab test and Solo Step Ab test) and Symbiotics Corporation (ASSURE Ab test) use a recombinant Ag to detect host Ab. The ASSURE Ab test uses an Ag (ie, DIr33) sequenced from Ag of microfilaria, L3 larvae, and adult worms. The HESKA Veterinary Lab Ab and Solo Step tests use recombinant Ag (ie, rHWAg1) that is sequenced from Ag found in microfilaria, L3 and L4 larvae, and adult worms. The Diagnostics Lab Ab test uses heartworm Ag extracted and purified from an equal weight of adult male and female heartworms.

A negative heartworm Ab test result suggests the following 3 possibilities: the cat is not infected with heartworms, the cat has an infection < 50 to 60 days old, or an insufficient amount of IgG Ab was detected against the Ag used in formulating the Ab test. On the basis of findings in our study, if a cat has clinical signs that suggest heartworm disease despite a negative heartworm Ab test result, we recommend that an alternative heartworm Ab test, a heartworm Ag test, thoracic radiography, or two-dimensional echocardiography be performed. Alternatively, the heartworm Ab test may be repeated in 2 to 3 months if clinical signs persist.

In our study, hemolysis prevented the reading of 3 Solo Step results. Overall, however, neither hemolysis nor lipemia appeared to affect test results with the following minor exception. Specificity of the Solo Step test (read after a 10-minute incubation) increased significantly with hemolyzed blood samples, and the sensitivity of this test (read after a 5-minute incubation) increased with lipemic blood samples, compared with unaffected (not hemolyzed and not lipemic) blood samples.

As reported by others, we found the specificity of heartworm Ag tests to be high, ranging from 98.1 to 99.4%. As expected, 1 cat with a single male worm had negative results on all Ab tests. However, another cat infected only with 2 male worms had positive results on all 4 Ag tests, and 2 cats with a single male worm had positive results on 1 of 4 heartworm Ag tests. Because the heartworm Ag tests detects proteins predominantly associated with the female reproductive tract, the most likely explanation is that these cats must have been infected with at least 1 female worm that was not found on necropsy examination. A less likely alternative is that the Ag tests cross-reacted with another parasitic Ag or that the male worm was secreting sufficient quantities of Ag detected by the tests.

Because a positive Ag test result is strongly associated with adult female heartworms, a positive result would not be expected until 6 to 7 months after infection. However, in experimentally infected cats, heartworm Ag test results were positive as early as 5 months after infection. McCall and others used canine heartworm Ag tests to detect heartworms in experimentally infected cats. These investigators found that the sensitivity of tests varied with the number of worms and ranged from 61.3 to 83.9%. Unfortunately, although they reported the total number of worms, the number of female versus male worms was not reported. McCall also states that positive heartworm Ag test results are expected in 33 to 40% of naturally infected cats and that positive results are dependent upon a number of factors including sex, age, and number of heartworms and to a lesser extent the size of the host. Although the number of cats that had positive necropsy results is small in our study, we found the sensitivity of feline heartworm Ag tests to range from 68.4 to 79%. In some instances, the sensitivities of the Ag test approached or exceeded the sensitivities of the Ab test. Results of our Ag testing are similar to those of Atkins et al who found positive Ag test results in 86% of heartworm infected cats. McCall et al evaluated the Ag tests performed by HESKA Lab and Diagnostics Lab in experimentally infected cats. They found HESKA Lab Ag test results were positive 6 to 9 months after infection in 63% of cats that had positive necropsy results and Diagnostics Lab Ag test results were positive in 100% of cats that had positive necropsy results 6 to 9 months after infection. Number of worms and sex of worms recovered from the experimentally infected cats in McCall et al’s study was not reported. Because our study involved natural infection, the duration of infection in our cats is unknown.

Dillon has suggested that Ag shedding may increase during periods of worm stress. In our study, the degree of Ag shedding by the worm could have increased in association with the host’s death, because
blood samples were not collected until after euthanasia. An increase in Ag shedding could have increased the detection rate of tests. It has not been determined what if any effect host death or euthanasia solution has on Ag test performance. Nelson et al reported ante-mortem and postmortem heartworm Ab test results in 98 shelter cats. They found 23.5% of cats had positive HESKA Lab Ab test results before death and 36.5% had positive HESKA Lab Ab test results after death. Unfortunately, time from euthanasia to postmortem blood collection was substantially longer (8 to 16 hours) than in our study. Antigen tests were not examined before death and were only performed in cats that had positive necropsy results.

Based on a theoretic heartworm infection prevalence of 5%, the positive-predictive values of these Ag tests ranged from 68.3% to 85.8%, all higher than the positive-predictive values of Ab tests (Table 3). Using a prevalence of 5%, the negative-predictive values of all tests were high. This results from the assumption that there is a low prevalence rate and the moderately good overall performance of tests (AUC-ROC, ranging from 65.3% to 88.5%; Tables 1 and 2). Even with a 50% sensitivity and 50% specificity, the negative-predictive value would be 95% when the prevalence is 5%.

The main limitation of our study was the reliance upon necropsy as the standard for determining heartworm status in cats. Because the propensity of cats to develop ectopic infections is high, gross examination of the vena cavae, right atrium, right ventricle, and pulmonary arteries (to a size of about 1 mm in diameter) will fail to identify some adult worms, and precardiac infections will be missed. However, the complexity involved in performing a thorough examination of thoracic and abdominal body cavities, the subcutaneous tissues, and the systemic arterial system and nervous system precludes this sort of study in most instances. In addition, because some tests detect Ab to immature as well as adult worms, cats can have positive Ab test results as early as 60 days after infection when no adult worms are found. In both situations, a positive test result would be termed a false-positive result, when gross examination is used as the definitive result.

Limiting the examination for heartworms to the areas previously mentioned prevents us from conclusively stating that the cats that had negative necropsy results with positive Ag or even positive Ab test results did not have heartworm infection. Cross-reactivity with gastrointestinal parasites was not assessed, because fecal examinations were not done. Because of difficulties in determining true infection rate (because of ectopic infections), researchers have turned to experimental infection to provide the best means for defining true specificity of heartworm tests. Unfortunately, many cats that are experimentally infected with D immitis have higher numbers of adult worms than naturally infected cats. So although there is guarantee of infection with experimental implantation of larvae, the simulation of natural infection with single sex infection or low worm burdens may be lacking. Implanting known numbers of adult heartworms into heartworm naive cats may improve this situation, but it does not mimic natural infection because the tissue migration phase is lacking. Additionally, cats in a natural setting are repeatedly exposed and, therefore, presumably repeatedly infected.

One limitation of tests used in our study is the technician’s confidence in distinguishing a positive from a negative test result. Having multiple people perform or read the results on identical samples and then determine a mean value from these results could minimize this factor. This was not done in our study nor was test repeatability examined. People performing the in-house tests were well educated and had been trained by the senior investigators or representatives from the manufacturers. Their skill level and understanding of test technique were thought to equal or exceed that of a technician performing these tests in a veterinary practice. We evaluated Solo Step Ab test at the recommended incubation time of 5 minutes and also after 10 minutes and 20 minutes. Our results indicate that the test performed best when a 20-minute incubation time was used. We did not evaluate other in-house tests using different incubation times and thus cannot comment on how other incubation times may have influenced other Ab or Ag test results.

Another limitation of our study was the small number of cats studied (330 cats) and the small number of cats that had positive necropsy results (19 cats). However, the number of cats in both categories is similar to or greater than the number of cats examined in other feline heartworm studies. Because of small number of cats with heartworm infections in our study, limited statistical power was attained. Thus we may not have been able to detect differences in test sensitivity smaller than the range of sensitivities we observed in each test alone and to detect differences in test sensitivities larger than the range of sensitivities we observed in the in the Ab/Ag pairs testing.

Although not necessarily a limitation of our study, it should be kept in mind that we used the simple mean of sensitivity and specificity as an overall indicator of test performance. Equal weights were assigned to the correct diagnosis of heartworm infection. Different weights for these indicators, as well as for positive- and negative-predictive value, would be appropriate under certain clinical situations. If these had been weighted so as to make one (ie, sensitivity or specificity) more important, the test performance would be different. For example, if a veterinarian cannot tolerate having false-positive results (eg, when heartworm infection must be excluded from a differential diagnosis list), then the importance of test specificity should be weighted more heavily. Alternatively, if further testing would automatically be performed on a healthy cat that had positive results, then a high false-positive rate would be more tolerable.

By using the mean of the sensitivity and specificity (AUC-ROC), an overall ranking of test performance was obtained for cats in our study. The DiroCHEK Ag test ranked the highest with a AUC-ROC of 88.5% (Table 2). Using this criterion, several other tests also ranked high. When an Ab test and Ag test were combined, the test pairs that ranked the highest included the Solo Step Ab test (incubated for 20 minutes) and either the DiroCHEK Ag test, SNAP Ag test, or the
HESKA Lab Ag tests (AUC-ROC, 90 to 92%). Many of the other combinations had only slightly lower AUC-ROC. Pairing of an Ag test with an Ab test does appear to improve diagnostic performance of serologic tests of heartworm infection.


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