Comparison of Serological Tests for the Detection of Natural Heartworm Infection in Cats

Serological tests were performed on 380 cats with necropsy-confirmed heartworm disease to compare the performance of currently available commercial laboratory and point-of-care heartworm serological tests in a heartworm-endemic area. Overall, antigen tests detected 79.3% to 86.2% of heartworm infections and were highly specific. Most cats with false-negative antigen tests had a single male worm. Antibody tests detected 62.1% to 72.4% of heartworm infections and had a wider range of false-positive results (1.4% to 19.1%) than antigen tests (0.3% to 2.0%). Serological tests for feline heartworm infection varied in diagnostic performance. Combining results from antigen and antibody tests achieved greater sensitivity than using either test alone. **J Am Anim Hosp Assoc 2004;40:376-384.**

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Introduction

Dirofilaria immitis is a common filarial nematode parasite of dogs transmitted by mosquitoes. Although awareness of heartworm infection and disease in cats has increased in the past decade, the first reported case of feline heartworm infection in the United States was in 1922.[1] Heartworm prevalence in cats depends upon the local infection rate of dogs, the definitive hosts.[1] Most necropsy studies within the southeastern United States demonstrate a prevalence in the feline population of 2.5% to 14%, which is approximately 5% to 20% of the local prevalence in dogs.[1-4]

Antemortem diagnosis of feline heartworm infection is often challenging. Tests for microfilaremia are positive in <20% of infected cats.[5,6] Radiographic findings may support a diagnosis of heartworm infection, but abnormalities are often subtle and nonspecific. Approximately half of cats with clinical signs associated with heartworm infection have normal thoracic radiographs.[7-10] Adult worms were identified in 40% to 56% of infected cats when echocardiography was performed by noncardiologists without a high index of clinical suspicion, and identifications were made in 64% to 78% when cardiologists performed echocardiograms.[11-15] Gross necropsy identification of fourth-stage (L4) and fifth-stage (L5) larvae or adult worms provides a definitive postmortem diagnosis of infection.

Serology for heartworm antigen and antibody has become an important antemortem diagnostic tool for detecting heartworm infection, and 18 different tests are currently licensed for use in cats and dogs. Antigen tests detect proteins shed by the parasite, so such tests may be useful in both dogs and cats, even if they are not developed specifically for use in cats. Antibody tests detect antibodies produced by the host in response to the parasite and are only useful in the species for which they are developed. Only female reproductive tract antigens are detected in heartworm antigen tests. Detection of female worms is useful in dogs because of their often large, mixed-gender worm burdens. However, in cats, low

worm burden and male-only heartworm infections reduce the quantity and type of antigens available for detection, resulting in a higher frequency of false-negative antigen test results.[5] Most cats exposed to heartworms react by producing antibody, but a positive antibody test result only indicates exposure to heartworms and not necessarily a current infection. Additionally, antibody is not detected in all infected cats.[16]

The development of serological tests specifically for cats and the availability of multiple point-of-care (i.e., in-house or cage-side) and reference laboratory testing options provide many choices for the serological diagnosis of heartworm in cats. The purpose of this study was to compare the performance of several currently available serological tests against necropsy results in a population of cats residing in a heartworm-endemic area.

Materials and Methods

The 380 cats used in this study (36% male, 64% female) were obtained following euthanasia at the Alachua County Animal Services facility in Gainesville, Florida. Cats were examined between March and June 1998 (n=80) and June to September 2001 (n=300).[17]

Gross necropsy of the thoracic cavity was performed within 2 hours of euthanasia. Blood from the thoracic vena cava was collected into serum separator tubes and separated by centrifugation. Serum aliquots were stored at -80˚C pending testing. Frozen serum storage time prior to testing varied from 1 to 36 months. The heart and lungs were removed from the thoracic cavity, and all cardiac chambers and pulmonary arteries were opened and inspected for the presence of adult worms. Heartworms found at necropsy were collected, counted, and sexed.

Personnel performing the serological tests were blinded to the necropsy findings and to the results of the other serological tests. The frozen serum samples were thawed from -80˚C to room temperature and then vortexed prior to antigen and antibody testing.

The three point-of-care immunochromatographic test kits for antigen detection used were IDEXX Laboratories Inc. SNAP Feline Heartworm Antigen Test Kit[a] (IDEXX antigen), SA Scientific Inc. CHAT Canine Heartworm Antigen Test Kit[b] (SA Scientific antigen), and Synbiotics Corp. DiroCHEK Canine Heartworm Antigen Test Kit[c] (Synbiotics antigen). Technology utilized involved a membrane-format enzyme-linked immunosorbent assay (ELISA) for the IDEXX antigen, lateral-flow immunochromatography for the SA Scientific antigen, and a microwell ELISA format for the Synbiotics antigen test.

For antibody detection, one point-of-care test kit, Synbiotics Corp. Witness FHW Feline Heartworm Antibody Test Kit[d] (Synbiotics antibody), was used. Antech Diagnostics[e] (Antech antibody) performed the other antibody test upon submission of serum samples to the reference laboratory. The Synbiotics antibody used lateral-flow immunochromatography, and Antech antibody used an immunofluorescent antibody assay. All point-of-care tests were performed according to manufacturers' instructions. All serological assays reported here were different from those performed on serum from 80 of the cats and published in an earlier article.[17]

The diagnostic performances of the five heartworm tests were assessed, using gross necropsy detection of heartworm as the positive control. The sensitivity, specificity, and corresponding 95% confidence interval for each test were calculated. In addition, Bayes' Rule was used to compute positive- and negative-predictive values for each test, given a projected heartworm prevalence of 1%, 5%, and 10%. All data was analyzed using Microsoft Excel Analyze It software.[f]

The McNemar test for comparison of correlated proportions was used to assess pair-wise differences among sensitivities and specificities for the five tests. To maintain an overall α=0.05, the Bonferroni procedure was used. Thus, for each pair-wise comparison, α was set at 0.005.

In addition to the overall performance of each test, combinations of antigen and antibody tests were assessed. Since there were three antigen and two antibody tests, there were a total of six possible antigen/antibody pairings. Each pairing was considered a single composite test (a positive test result on either test was considered positive for the pair), and results were compared to necropsy standard results. Sensitivity and specificity of paired antigen and antibody tests were compared to single antigen or antibody tests.

Results

Of the 380 cats necropsied, 29 had adult heartworms found within their cardiopulmonary vasculature. A total of 50 adult heartworms were recovered, consisting of 22 males and 28 females, nine of which were gravid. The number of adult worms per cat ranged from one to four, with a mean and median of 1.7 and 1.0, respectively. A single worm was recovered in 51.7% (15/29) of infected cats, with 17.2% (5/29) having a single male worm. Single sex infections, which included cats infected with one or more worms of the same sex, were present in 62.1% (18/29) of cats. The single sex infections were composed of 66.7% (12/18) all-female and 33.3% (6/18) all-male worms [Table 1].

The IDEXX antigen test produced positive results in 7.9% (30/380) of the cats. This test correctly identified 23 of 29 infected cats, resulting in a sensitivity of 79.3%. Additionally, 344 of 351 heartworm-free cats were correctly identified, resulting in a specificity of 98.0%. The six heartworm-infected cats that tested negative had single worm infections, five of which were single male worm infections [Table 2].

The SA Scientific antigen test produced positive results in 6.3% (24/380) of the cats. This test correctly identified 23 of 29 infected cats, resulting in a sensitivity of 79.3%. Additionally, 350 of 351 heartworm-free cats were correctly identified, resulting in a specificity of 99.7%. The six heartworm-infected cats that tested negative had single worm infections, five of which were single male worm infections.

The Synbiotics antigen test produced positive results in 7.4% (28/380) of the cats. This test correctly identified 25 of 29 infected cats, resulting in a sensitivity of 86.2%. Additionally, 348 of 351 heartworm-free cats were correctly

Table 1

Necropsy and Serological Test Findings in 29 Heartworm-Infected Cats

Case No.	Breed*	Sex†	Total Worm Burden	Male Worms	Female Worms	IDEXX Antigen[a]	SA Scientific Antigen[b]	Synbiotics Antigen[c]	Synbiotics Antibody[d]	Antech Antibody[e]
1	DSH	F	1	0	1	+	+	+	+	-
2	DSH	F	3	1	2	+	+	+	+	+
3	DSH	F	2	1	1	+	+	+	-	-
4	DSH	M	2	1	1	+	+	+	-	+
5	DSH	M	3	2	1	+	+	+	-	+
6	DSH	M	1	1	0	-	-	-	+	+
7	DSH	M	1	1	0	-	-	+	+	+
8	DSH	F	2	1	1	+	+	+	+	+
9	DSH	F	1	0	1	+	+	+	-	-
10	DSH	F	3	2	1	+	+	+	+	+
11	DSH	M	3	0	3	+	+	+	-	-
12	DSH	F	2	1	1	+	+	+	+	+
13	DSH	M	2	1	1	+	+	+	-	+
14	DLH	M	1	0	1	+	+	+	+	+
15	DSH	M	1	0	1	+	+	+	-	+
16	DSH	M	2	1	1	+	+	+	+	-
17	DSH	F	3	1	2	+	+	+	+	+
18	DSH	M	1	0	1	-	-	+	+	+
19	DSH	M	2	2	0	+	+	+	+	+
20	DSH	M	1	1	0	-	-	-	+	+
21	DSH	CM	1	0	1	+	+	+	+	+
22	DLH	CM	2	0	2	+	+	+	+	+
23	DSH	M	1	0	1	+	+	+	-	+
24	DSH	M	1	1	0	-	-	-	+	+
25	DSH	F	1	0	1	+	+	+	-	-
26	DSH	SF	1	1	0	-	-	-	-	-

Continued on next page

Table 1 (cont'd)

Necropsy and Serological Test Findings in 29 Heartworm-Infected Cats

Case No.	Breed*	Sex†	Total Worm Burden	Male Worms	Female Worms	IDEXX Antigen[a]	SA Scientific Antigen[b]	Synbiotics Antigen[c]	Synbiotics Antibody[d]	Antech Antibody[e]
27	DSH	F	4	3	1	+	+	+	+	+
28	DLH	SF	1	0	1	+	+	+	+	+
29	DSH	F	1	0	1	+	+	+	-	-
	Total	29	50	22	28	23	23	25	18	21

* DSH=domestic shorthair; DLH=domestic longhair
† F=female; M=male; CM=castrated male; SF=spayed female
[a] IDEXX antigen=SNAP Feline Heartworm Antigen Test Kit; + = positive; - = negative
[b] SA Scientific antigen=CHAT Canine Heartworm Antigen Test Kit
[c] Synbiotics antigen=DiroCHEK Canine Heartworm Antigen Test Kit
[d] Synbiotics antibody=Witness FHW Feline Antibody Test Kit
[e] Antech antibody=Feline Heartworm Antibody Test

Table 2

Diagnostic Performance of Heartworm Antigen and Antibody Tests in 380 Cats

Serological Test	% Sensitivity	% Specificity	True Positives	False Positives	True Negatives	False Negatives
IDEXX Snap antigen	79.3	98.0	23	7	344	6
SA Scientific CHAT antigen	79.3	99.7*	23	1	350	6
Synbiotics DiroCHEK antigen	86.2	99.1	25	3	348	4
Synbiotics Witness antibody	62.1	98.6	18	5	346	11
Antech Diagnostics antibody	72.4	80.9†	21	67	284	8

* SA Scientific CHAT antigen specificity was significantly higher when compared to IDEXX Snap antigen (P=0.031).
† Antech Diagnostics antibody specificity was significantly lower when compared to the other tests (P=0.001).

Table 3

Diagnostic Performance of All Possible Heartworm Antigen and Antibody Test Pairs in 380 Cats

Antigen Test	Antibody Test	% Sensitivity	% Specificity	True Positives	False Positives	True Negatives	False Negatives
Synbiotics antigen	Antech antibody*	96.6	80.6	28	68	283	1
IDEXX antigen	Antech antibody	96.6	79.8	21	71	280	1
SA Scientific antigen	Antech antibody	96.6	80.9	28	67	284	1
Synbiotics antigen	Synbiotics antibody	89.7	98.0	26	7	344	3
IDEXX antigen	Synbiotics antibody	86.2	96.9	25	11	340	4
SA Scientific antigen	Synbiotics antibody†	86.2	98.6	25	5	346	4

* All pairings that include Antech Diagnostics antibody had significantly lower specificity when compared to pairs that included Synbiotics antibody (P<0.05).
† Synbiotics antibody paired with SA Scientific antigen was significantly more specific when compared with Synbiotics antibody paired with IDEXX antigen (P<0.05).

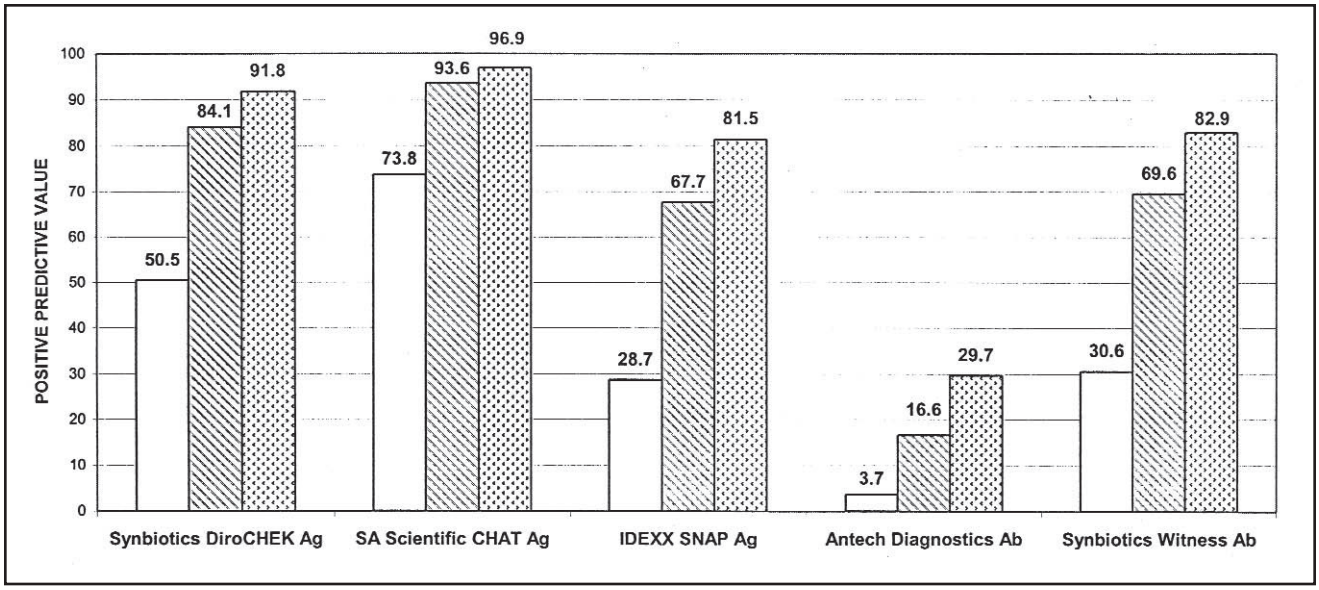

***Figure 1*—**Positive predictive values (reliability of a positive test result) of heartworm antigen (Ag) and antibody (Ab) assays for theoretical heartworm prevalences of 1%, 5%, or 10% within a population tested. Positive predictive values (PPV) vary between assays and increase markedly as true prevalence increases (PPV 1% [blank white columns]; PPV 5% [diagonal striped columns]; PPV 10% [dotted < and > columns]).

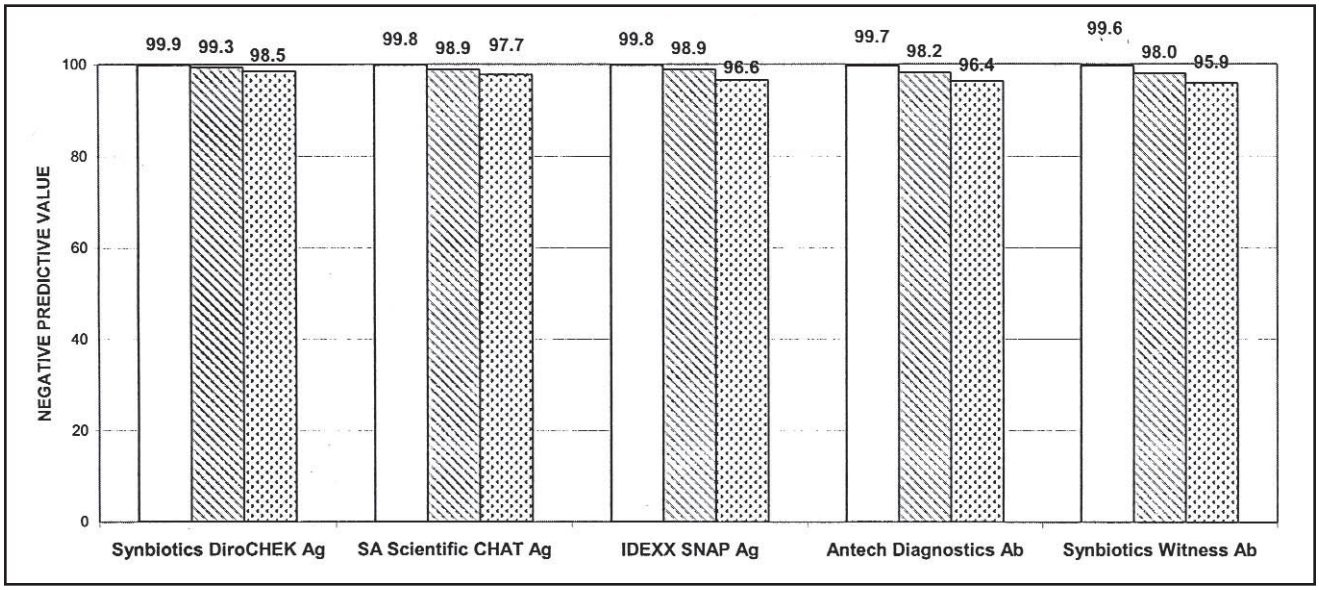

***Figure 2*—**Negative predictive values (reliability of a negative test result) of heartworm antigen (Ag) and antibody (Ab) assays for theoretical heartworm prevalences of 1%, 5%, or 10% within a population tested. Negative predictive values (NPV) vary slightly between assays and change little as true prevalence changes (PPV 1% [blank white columns]; PPV 5% [diagonal striped columns]; PPV 10% [dotted < and > columns]).

identified, resulting in a specificity of 99.1%. The four heartworm-infected cats that were falsely negative had a single male worm.

Collectively, the IDEXX antigen, SA Scientific antigen, and Synbiotics antigen tests detected 79.3% to 86.2% of the heartworm infections and were highly specific. False-positive antigen results ranged from 0.3% to 2.0%. False-negative antigen results ranged from 13.8% to 20.7% [Table 2]. Six (20.7%) cats accounted for all 16 false-negative results produced collectively by the Synbiotics antigen (n=4), IDEXX antigen (n=6), and SA Scientific antigen (n=6) tests. All of the false-negative antigen results occurred with single worm infections, 83.3% (5/6) of which were single male worms. Two heartworm-infected cats had negative results with two antigen tests, and four heartworm-infected cats were negative with all three antigen tests. There were no significant differences in sensitivity (true positives) between the various antigen tests ($P \geq 0.18$). Of the 351 heartworm-free cats, seven cats accounted for all 11 false-positive results produced collectively by the Synbiotics

antigen (n=3), IDEXX antigen (n=7), and SA Scientific antigen (n=1) tests. Four of the cats had false-positive results on one antigen test, two cats had false-positive results on two antigen tests, and only one cat had a false-positive result on all three antigen tests. The SA Scientific antigen was more specific for adult heartworm infections than the IDEXX antigen test (P=0.031). All other antigen tests had a similar number of false positives (specificity) (P≥0.125).

The Antech antibody test produced positive results in 23.2% (88/380) of the cats. This test correctly identified 21 of 29 infected cats, resulting in a sensitivity of 72.4%. Additionally, 284 of 351 heartworm-free cats were correctly identified, resulting in a specificity of 80.9%. Of the eight cats that were falsely negative, three had multiple worm infections, and five had single worm infections.

The Synbiotics antibody test produced positive results in 6.1% (23/380) of the cats. This test correctly identified 18 of 29 infected cats, resulting in a sensitivity of 62.1%. Additionally, 346 of 351 heartworm-free cats were accurately identified, resulting in a specificity of 98.6%. Of the 11 cats that were falsely negative, three had multiple worm infections and eight had single worm infections.

Collectively, the Antech and Synbiotics antibody tests detected 62.1% to 72.4% of the heartworm infections. False-positive antibody results ranged from 1.4% to 19.1%. False-negative antibody results ranged from 27.6% to 37.9% [Table 2]. Of the 29 cats identified as heartworm infected at necropsy, 51.7% (15/29) had at least one false-negative antibody result. Eleven cats had a false-negative result on one heartworm antibody test, and four cats had a false-negative result on both antibody tests. There was no significant difference in sensitivity (true positives) between the antibody test results. Together, the Synbiotics antibody and Antech antibody tests had false-positive results in 20.5% (72/351) of cats that were negative for adult heartworm infection at necropsy. The Antech antibody test had a significantly higher (P=0.001) rate of false-positive results (19.1%, 67/351) compared to the Synbiotics antibody test (1.4%, 5/351).

For the various tests studied, positive predictive values and negative predictive values were calculated for a theoretically low (1%), moderate (5%), and high (10%) prevalence of feline heartworm infection within a population. Depending upon the prevalence of infection within the population, positive predictive values of the antigen tests ranged from 28.7 to 96.9, and negative predictive values ranged from 96.6 to 99.9. Positive predictive values of the antibody tests ranged from 3.7 to 82.9, and negative predictive values ranged from 95.9 to 99.7 [Figures 1, 2].

The sensitivities (true positives) of the antigen-antibody test pairs ranged from 86.2% to 96.6% and were not significantly different from each other or from antigen tests used alone (P≥0.25) [Table 3]. However, the sensitivity of all antigen-antibody pairs was higher (P≤0.016) than any antibody test used alone.

The specificities (true negatives) of the antigen-antibody test pairs ranged from 79.8% to 98.6% [Table 3]. The IDEXX antigen-Synbiotics antibody pairing resulted in a lower specificity (96.9%) compared to the SA Scientific antigen-Synbiotics antibody pairing (P=0.03). The lowest specificities (79.8% to 80.9%) occurred when any of the three antigen tests were paired with the Antech antibody test (P=0.001) [Table 3]. When antigen and antibody tests were paired, specificity was frequently lower (P≤0.031) when compared to the specificity of single antigen tests used alone.

Discussion

The purpose of this study was to evaluate the performance of several currently available heartworm antigen and antibody tests in cats with necropsy-confirmed, natural heartworm infections. Although the number of heartworm-infected cats was low (29/380), to the authors' knowledge this is the largest serological performance study in naturally infected cats. The recovered worm burden and worm sex distribution were similar to previous reports.[1,6,17-20] Low worm burdens are a common finding in feline heartworm infections, and they are important to recognize, because even a single adult worm can cause severe disease and death in cats.

Each of the five antigen and antibody tests evaluated in this study identified a majority of the heartworm-infected cats. There were no significant differences in test sensitivity between the tests, but specificity did vary. Higher sensitivity for detecting heartworm infection was achieved with some combinations of antigen-antibody tests, but the results were generally accompanied by decreased specificity.

Serological results in this study correlated with worm burden. Every cat with multiple worm infections was positive on all three of the antigen tests, whereas only 60% of the cats with a single worm infection were positive on all antigen tests. Cats infected with multiple worms had two positive antibody results in 64.3% of the cases, whereas 33% of cats infected with only one worm had positive antibody results on both tests. False-negative antigen or antibody test results were less common (17.1%) in cats with multiple worms than in cats with a single worm (82.9%).

The sensitivities of the antigen tests in this study were higher than in many previous reports of both natural and experimental infections.[8,16,18-23] Reasons for improved antigen test sensitivity in this study were unknown but may have indicated improved test technology. The only infected cats that were not identified by any of the antigen or antibody tests had single male worm infections. One cat infected with two male worms was detected by all three antigen tests, whereas the five cats with single male worms were missed by 93.3% (14/15) of the antigen tests. Although heartworm antigen tests are designed to detect female worm proteins, all three antigen tests yielded positive results in the two cats from which only one male worm was recovered. Possible explanations for these results included the presence of a female worm at an undetected ectopic location, the recent death of a female worm, or test sensitivity to male as well as female worm proteins.

Test accuracy and predictive values vary depending on the true prevalence of disease within a population.

Necropsy-confirmed heartworm prevalence in cats ranges from 0% in areas with low endemic heartworm infection in dogs to 14% in areas with high canine infection rates, particularly in the southeastern United States.[1,4,24-26] True prevalence also increases when subgroups of high-risk cats, such as cats with cardiopulmonary clinical signs, are selected for testing. The positive predictive values and negative predictive values change as true prevalence changes. The negative predictive values for all of the antigen and antibody tests were very high and exceeded the positive predictive values of all the assessed tests. This implies that all of the tests are more accurate for ruling out heartworm infection than they are for diagnosing heartworm infection. The predictive values also varied among the tests. Based on a theoretical prevalence of 5% to 10%, the low positive predictive value of one of the antibody tests (Antech) suggested that cats with a positive antibody test result were unlikely to be truly infected. The remaining tests all had a relatively high positive predictive value at a prevalence rate of 5% to 10%, suggesting that most cats with a positive test result were truly infected. With a theoretical prevalence of feline heartworm infection of only 1%, which would be common in regions of the country with low heartworm prevalence in dogs, positive predictive values varied widely, but negative predictive values were very high for all tests.

A positive antigen test suggests one of the following scenarios: current heartworm infection, a recently cleared infection, or the false reporting of the presence of antigen.[17] A negative antigen test result suggests the absence of heartworm infection, an infection that is <5 to 6 months old, or a false-negative result.[6] A positive antibody test suggests one of the following scenarios: current heartworm infection with larval or adult stages, previous exposure with the persistence of antibody, or the false reporting of the presence of antibody.[6] A negative antibody test result suggests the absence of exposure to heartworms, an infection that is <60 days old, failure of the cat to produce detectable antibodies, or a false-negative result.[6,26]

In the past, antibody tests were reported to be more sensitive, but less specific, than antigen tests in cats.[6] Based on this assumption, antibody tests are currently recommended as screening tests for heartworm infection.[6] Antigen tests have been used for confirmation of infection in antibody-positive cats, because they were believed to be less sensitive but more specific. However, in the study reported here, as well as in a previous study of other antigen and antibody tests, the sensitivities of the heartworm antigen tests were similar to or higher than the antibody tests for the detection of adult heartworms in naturally infected cats.[8,16,17,19-23] Overall, the three antigen tests tended to perform better than the two antibody tests for the detection of natural heartworm infections in cats. The combination of an antigen and antibody test improved overall sensitivity compared to the use of individual antibody tests alone. The increased sensitivity observed in antigen-antibody pairs compared to antigen tests alone did not reach statistical significance. These results suggested that simultaneous use of both antigen and antibody tests should replace previous recommendations for testing cats with an antibody test alone.

This study had several limitations. The shelter cat population used in this study did not necessarily represent the pet cat population for which the tests are targeted. Variation in exposure to heartworm vectors and resistance to heartworm infection between stray and pet cats may have impacted the prevalence of infection within these populations. The number of naturally infected cats in this study was low. Larger numbers of infected cats may be obtained in experimental studies in which cats are inoculated with larvae or transplanted with adult worms; however, experimental studies fail to mimic the natural host-parasite relationship and may lead to variation in antigen and antibody responses.[16,18,21]

Similar to other studies, this study used necropsy findings as the positive indicator of a heartworm infection. The major limitation of this indicator was the inability to detect migrating L4 and L5 larvae, which are capable of producing both clinical signs and positive antibody results. A necropsy limited to the thoracic cavity may have also missed ectopic adult worms in sites outside the cardiopulmonary vasculature, such as the subcutis, body cavities, hepatic and renal vessels, and central nervous system.[11,16,28-30] In addition, with this study design, it was not possible to determine if necropsy-negative cats had recently recovered from infection. Cats that have recovered from heartworm infection may have persistent antigen and antibody present for several months following elimination of the parasite.[6]

Whereas heartworm serological tests are designed for use in live animals, this study evaluated serum samples collected shortly after euthanasia. Changes in quality or quantity of antigen and antibody after death of the host may affect test performance. One study has reported that heartworm antigen and antibody test results in cats prior to euthanasia differed slightly from results of blood samples acquired from the same cats 8 to 16 hours after euthanasia.[20] To the authors' knowledge, no study has investigated changes in antigen and antibody results in blood samples collected within 2 hours of death. It is also possible that the sampling of blood directly from the thoracic vena cava may contain higher amounts of heartworm antigen compared to blood samples acquired from peripheral veins, thus falsely increasing the reported sensitivity of the antigen tests. Lastly, the effects of long-term storage of frozen serum samples on feline antigen and antibody heartworm test performance are unknown. Storage of canine serum samples at -70°C for several years, however, has not affected performance of heartworm antigen tests.[31]

Conclusion

Serological tests for heartworm detection in cats vary in overall diagnostic performance. A negative test does not preclude adult heartworm infection, nor does a positive test indicate a definitive diagnosis. Despite similarities in sensitivity, antigen assays of this study outperformed one of the two antibody tests in specificity and positive predictive value based on a necropsy diagnosis of heartworm infection.

Worm burden characterized by single male infections frequently resulted in false-negative antigen and antibody tests in naturally infected cats. Combining results from antigen and antibody tests improved detection of heartworm infection compared to using either test alone, but it also resulted in more false-positive results.

[a] SNAP Feline Heartworm Antigen Test Kit; IDEXX Laboratories, Inc., Westbrook, ME 04092
[b] CHAT Canine Heartworm Antigen Test Kit; SA Scientific Inc., San Antonio, TX 78240
[c] DiroCHEK Canine Heartworm Antigen Test Kit; Synbiotics Corp., San Diego, CA 92128
[d] Witness FHEARTWORM Feline Antibody Test Kit; Synbiotics Corp., San Diego, CA 92128
[e] Feline Heartworm Antibody Test; Antech Diagnostics, Farmingdale, NY 11735
[f] Microsoft Excel Analyze It software; Analyze-It Software, Ltd. 1997, Version 1.62, Leeds, England

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