

Diagnosing Feline Heartworm Disease

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Introduction

Heartworm disease can cause serious illness in cats that is potentially life-threatening or fatal, and is difficult to manage since there is no approved treatment. The objectives of this paper are to better understand feline heartworm infection as it relates to diagnostic options, and report the accuracy of the VETSCAN® Heartworm and WITNESS® FFH Rapid Tests in cats using USDA submission data.

Prevalence

Heartworm has been reported in all 50 states, and the number of reported cases has been increasing nationwide over the last decade.¹ The true prevalence of feline heartworm infection is likely underestimated due to: (1) lack of awareness and testing; (2) diagnostic limitations of currently available tests; (3) non-specific clinical signs; and (4) lack of confirmation of infection in many clinically affected cats. As the potential for heartworm infection continues to rise in association with suboptimal compliance with regular preventive regimens, widespread transport of infected dogs, and environmental changes that encourage mosquito vectors, it is important to screen cats regularly for heartworm infection and institute routine preventive use.

In feline infections with adult worms, worm burdens are typically low.

In feline heartworm infections, just one worm can produce severe, sometimes fatal, disease.

Feline Heartworm Disease²

The heartworm lifecycle in cats starts with infective third-stage larvae, which are deposited on the skin by infected mosquitoes, where they penetrate the subcutaneous tissues. As they mature to fourth-stage larvae, they stimulate a potent inflammatory response in the

pulmonary tissues. This immune response is responsible for the typical signs of feline heartworm disease - coughing, wheezing and dyspnea, similar to idiopathic feline asthma, and is commonly known as HARD (Heartworm Associated Respiratory Disease). HARD is the most common clinical presentation of feline heartworm infection and is entirely in response to larval heartworm infection; it does not occur in every infected cat and some cats are asymptomatic. In some infected cats, larvae progress to young adult worms in the pulmonary arteries, and can develop into mature adults in the right side of the heart. When mature adult worms die, internal antigens, previously 'unseen' by the cat's immune system, are exposed as the worms disintegrate. This can result in an acute shock-like reaction known as the Acute Death Syndrome, which is usually fatal.

Feline Heartworm Diagnosis

Because of its unique pathophysiology, heartworm infection in cats is divided clinically into HARD and the Acute Death Syndrome, each caused by the immune response to a different lifecycle stage, and each detected using different diagnostic techniques. Larval heartworm infection produces a robust antibody-mediated response in infected cats; these antibodies can be detected using heartworm antibody tests from as early as two months after initial infection. Since detectable antibodies can persist for months to years, antibody tests indicate exposure to heartworm larvae and not necessarily current infection. However, all test results should be interpreted together with the history of routine

<p>heartworm preventive administration to more accurately assess the risk of current infection.</p>	<p>Most adult infections in cats consist of a low worm burden of less than six adult worms; a typical infection consists of one to two worms, and single sex infections are common². Since heartworm antigen tests are designed to detect antigen from the uterus of adult female worms, they are often relatively insensitive due to low circulating antigen concentrations and single-sex infections, but positive results can occur from 5.5 to 8 months after initial infection. Additionally, antigen-antibody complexes can reduce the amount of unbound antigen available for test capture. For all of these reasons, a negative antigen test cannot be used to rule out current infection.² If it occurs, microfilaremia in feline infections is very rare and transient, because microfilariae are subject to immune attack and they cannot be produced in single-sex infections.</p>	<p>USDA submission. The VETSCAN study investigated the heartworm infection status of 380 cats that were euthanized at an animal shelter in Alachua County, Florida. Necropsy was used as the reference standard test and the thoracic cavity, heart, and lungs were dissected and examined for the presence of adult heartworm. Serum was assayed for heartworm antigen using the VETSCAN Heartworm Rapid Test. The VETSCAN Heartworm Rapid Test correctly identified 23/29 adult heartworm positive cats and 350/351 negative cats (Table 1). Based on this study, the sensitivity and specificity of the VETSCAN Heartworm Rapid Test was 79.3% and 99.7%, respectively when used to test feline sera for adult heartworm infection. The six heartworm-infected cats that tested negative on the VETSCAN Heartworm Rapid Test each had a single adult worm (n=5 male worms and n=1 female worm).</p>
<p>Since heartworm diagnostics detect different life cycle stages, multiple diagnostic modalities increase the likelihood that infection will be detected.</p> <p>The specificity of the VETSCAN Heartworm Rapid Test in cats was 99.7%, published in 2004 JAAHA study.</p> <p>In cats, maximum diagnostic sensitivity can be achieved by using antigen and antibody serology together to evaluate cats for heartworm infection. Thoracic radiographs and echocardiography can also be useful ancillary diagnostic aids.¹ Since the spectrum of clinical presentations is broad, from asymptomatic to fatal infections, transport of infected dogs from endemic areas can rapidly change local heartworm risk in previously low prevalence areas, and indoor-only housing status does not protect cats from exposure, the American Heartworm Society recommends routine screening for feline heartworm infection.</p> <p>VETSCAN Study Design and Results³</p> <p>This data was used with permission for</p>	<p>WITNESS Study Design and Results⁴</p> <p>In the WITNESS FFH study, banked feline serum or plasma samples from reference and research labs were used. Samples that tested heartworm-positive (n=81) and heartworm-negative (n=125) using DiroCHEK® antigen ELISA as the reference method were assayed to estimate the diagnostic sensitivity and specificity of WITNESS FFH and IDEXX SNAP® Feline Triple Test. Visual analysis and spectrophotometer analysis were used to determine whether the sample was DiroCHEK® antigen ELISA positive or negative. Compared to the reference standard, the sensitivity and specificity of the WITNESS FFH heartworm antigen test were 91.4% and 99.2%, respectively (Table 2). The SNAP Feline Triple Heartworm test had similar diagnostic accuracy, with a sensitivity of 93.8% and a specificity of 98.4% (Table 3).</p>	

Table 1. Sensitivity and specificity of VETSCAN Heartworm vs necropsy³

N=380	Reference Method Positive	Reference Method Negative
VETSCAN Heartworm Positive	23	1
VETSCAN Heartworm Negative	6	350

Sensitivity = 79.3% (95% CI: 59.7-91.3%)
Specificity = 99.7% (95% CI: 98.2-99.9%)

Table 2. Sensitivity and specificity of WITNESS FFH vs DiroCHEK antigen ELISA⁴

N=206	Reference Method Positive***	Reference Method Negative
WITNESS FFH Positive	74	1
WITNESS FFH Negative	7	124

Sensitivity = 91.4% (95% CI: 83.8-96.1%)
Specificity = 99.2% (95% CI: 96.3-99.9%)

Study No. D886Z-US-17-043

Table 3. Sensitivity and specificity of SNAP Feline Triple vs DiroCHEK antigen ELISA⁴

N=206	Reference Method Positive***	Reference Method Negative
SNAP Feline Triple Positive	76	2
SNAP Feline Triple Negative	5	123

Sensitivity = 93.8 % (95% CI: 87.0%-97.6%)
Specificity = 98.4% (95% CI: 95.0%-99.7%)

Study No. D886Z-US-17-043

*** vs DiroCHEK antigen ELISA

Low worm burdens, the presence of antigen-antibody complexes, immature female worms, and/or all-male infections can contribute to lower sensitivity for feline heartworm antigen testing.

The VETSCAN Heartworm Rapid Test and WITNESS FFH are specific tests that can be used in conjunction with other clinical information to diagnose feline heartworm infection rapidly and accurately.

Discussion

When comparing studies reporting head-to-head comparisons between point-of-care tests, careful interpretation is required, because differences in key factors such as sample type, population studied, and reference standards used can produce substantial differences in the results obtained. The VETSCAN and WITNESS studies reported here used necropsy and DiroCHEK® antigen ELISA as the reference method, respectively. In both studies, sensitivity was lower than specificity, supporting the American Heartworm Society recommendation that a negative antigen test should not be used to rule out feline heartworm infection. Importantly, cats should receive a monthly heartworm preventative that has been demonstrated safe in heartworm positive cats (or cats of unknown heartworm infection status). However, given the high specificity of all point-of-care antigen tests studied in feline specimens, a positive result is considered to be confirmation of an adult infection. Further diagnostics, which could provide relevant clinical information, include: heartworm antibody serology to detect the humoral response to larval heartworm infection, thoracic radiography and echocardiography.

Conclusion

The VETSCAN® Heartworm Rapid Test and WITNESS FFH Rapid Test are USDA licensed and approved for use in cats for the detection of *Dirofilaria immitis* antigen in feline whole blood, plasma, or serum. Screening using both antigen and antibody tests is recommended by the American Heartworm Society to aid in the diagnosis of a suspected active heartworm infection. The American Heartworm Society also recommends that cats receive year-round protection against heartworm infection, ideally with a preventative proven safe in heartworm-infected cats.²

References

1. Jason Drake and Scott Wiseman, Increasing incidence of *Dirofilaria immitis* in dogs in USA with focus on the southeast region 2013-2016 Parasites & Vectors (2018) 11:39 DOI 10.1186/s 2. American Heartworm Society, Summary of the Current Feline Guidelines for the Prevention, Diagnosis, and Management of Heartworm Infection in Cats. Feline Heartworm Guidelines Summary, <https://www.heartwormsociety.org/images/pdf/Feline-Guidelines%20Summary.pdf>, Published 2014, Accessed February 28, 2018, 3. Paul Berdoulay, Julie K. Levy, Patti S. Snyder, Michael J. Pegelow, Jennifer L. Hooks, Larissa M. Tavares, Nicole M. Gibson, and Marc E. Salute (2004) Comparison of Serological Tests for the Detection of Natural Heartworm Infection in Cats. Journal of the American Animal Hospital Association: September/October 2004, Vol. 40, No. 5, pp. 376-384. 4. Data on file, Study Report No. D886Z-US-17-043, Zoetis, Inc.