Although highly effective methods are available to prevent infection in domestic dogs, *D. immitis* has continued to spread throughout the U.S. Recent estimates of prevalence in dogs presented for veterinary care, based on a national clinic-based survey, range from 0% in a few Western and Midwestern U.S. areas to almost 7% in some regions of the southeastern states along the Mississippi River and the Gulf Coast.1 Factors influencing the spread of canine heartworm (CHW) include:

- Increased movement of domestic dogs across the U.S.
- Fewer mosquito and vector control programs in response to concerns about pesticide safety
- Lack of compliance with prevention programs
- Increased infection of nondomestic canid reservoir hosts, such as the American coyote.2

**DIAGNOSIS OF INFECTION**

In-clinic diagnosis of CHW infection is typically accomplished through detection of circulating microfilaria or soluble CHW antigen, and is dependent upon allowing adequate time from potential infection date to test date to account for the prepatent period (see *Life Cycle of Heartworms*, page 32). Because of this lag time, a general rule of thumb is to wait until a dog is 7 months of age before testing for the first time.4

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**Diagnosis of Canine HEARTWORM Infection**

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*Dirofilaria immitis*, the causative agent of canine heartworm disease, induces significant cardiopulmonary pathology in dogs in the United States and worldwide.
HEARTWORM HOSTS

While dogs are considered to be definitive hosts for heartworms, *D immitis* may infect many different species, including indigenous canids, such as wolves, foxes, and coyotes; many species of felids; and occasional incidental hosts, such as deer, ferrets, raccoons, horses, and primates.

The noncanid species are typically dead-end hosts; however, even a single heartworm in some animals may cause significant pulmonary disease, especially in cats. In humans, dying adult worms are associated with the development of pulmonary granulomas, which may be misdiagnosed as lung cancer on thoracic radiographs.
When to Test
Periodic testing, usually yearly, is recommended for all dogs, and it is advisable to retest whenever changing chemoprophylaxis agents or starting prophylaxis after a period of non-compliance. When changing heartworm preventive medication, additional testing 4 months after switching products is required to evaluate the efficacy of the original product. This process is especially important in light of recent suspicions of growing CHW resistance to macrocyclic lactones in some regions of the U.S. (see Prophylaxis Update).

Microfilaria Detection
Microfilaria may be visualized in whole blood using a direct smear technique or from blood concentrated using a modified Knott’s test or membrane filtration. Compared to other methods of heartworm detection, blood smears and concentration techniques are relatively insensitive; therefore, a test should not be considered negative until at least 1 mL of blood has been examined. However, the sensitivity of the modified Knott’s test is greater than that of the direct smear method when samples contain < 50 microfilaria per 1 mL of whole blood and the test provides enhanced ability to distinguish *D immitis* from other canine microfilaria.

Microfilaria tests are now viewed as supplemental assays, secondary to the more sensitive heartworm antigen tests. Microfilaria detection can be used to:
• Validate a positive antigen test
• Determine if the dog is a potential reservoir for infection
• Identify dogs at risk for severe reaction to treatment with microfilaricides.

Limitations of microfilaria tests include:
• The fact that about 20% of heartworm-infected dogs are not microfilaremic (occult infection), usually because only 1 sex of worm is present or the infection is prepatent.
• The use of macrolide preventives may result in clearance of microfilaria from the blood of treated dogs after several months of use.

LIFE CYCLE OF HEARTWORMS
1. The life cycle of a patent CHW infection starts with introduction of L3 larvae from an infected mosquito into the canid host.
2. In the dog, the larvae mature into L4 larvae and then into adult worms that localize in the pulmonary arteries and occasionally in the right cardiac ventricle.
3. Following a prepatent period of 6 to 7 months, microfilariae are produced via sexual reproduction and are released into the dog’s bloodstream, where they may remain viable for months to years.
4. Mosquitoes feeding on the infected host ingest the microfilaria, which then migrate to the Malpighian tubules where they mature into first-stage larvae.
5. Ultimately, the larvae mature to the L3 stage in the salivary glands of the mosquito and become infective, thus completing the heartworm life cycle.

PROPHYLAXIS UPDATE
Chemoprophylaxis is the primary weapon available to prevent heartworm infections in individual animals and controls spread of this parasite by reducing infections in the primary reservoir population. Macro cyclic lactones (ivermectin, moxydectin, selamectin, and milbemycin oxime) are the most commonly used heartworm preventives. The American Heartworm Society’s current guidelines for control of CHW infections in dogs include:
• Begin CHW prophylaxis in puppies as early as possible, usually before 8 weeks of age.
• Year-round chemoprophylaxis (even in areas where cold weather interrupts the CHW life cycle) is highly recommended to increase compliance.
• Although collies and some other dogs are sensitive to macrocyclic lactones, commercially available products for dogs used at the recommended doses are considered to be safe in all breeds.
• Reports of lack of efficacy of macrocyclic lactones in some areas of the U.S. are being investigated; currently, there is no strong evidence of widespread emergence of resistant strains of CHW. Lack of treatment and lack of compliance are of much greater concern.

The issue of apparent lack of efficacy is yet to be resolved. A recent report detailed genetic changes in some populations of heartworms that may confer greater resistance to chemoprophylactic agents. Whether or not these changes translate to the emergence of macrocyclic lactone-resistant strains of heartworms in endemic populations remains to be seen.
Antigen Detection
Enzyme-linked immunosorbent assay (ELISA) technology (Figure 1) was used in the first in-clinic CHW antigen tests; however, several current in-clinic CHW antigen tests are immunochromatographic (ICT) assays (Figure 2).

Even though they are more sensitive than microfilaria detection, CHW antigen tests:
• May give false negative results in animals that are infected only with male worms or with very few female worms; antigen testing fails in this case because the monoclonal antibodies used in the tests primarily detect female-specific antigens.
• Are not always precise with regard to correlation between the amount of heartworm antigen in the blood and the number of adult female worms.

Commercial CHW antigen tests evaluated during 2001 to 2002 were found to reliably detect at least 84% of CHW infections in which 3 or more female adult worms were present, but sensitivity decreased when fewer than 2 adult females were present.8,9

DNA Amplification
In addition to detection of microfilaria and/or heartworm antigen, other tests may be useful in diagnosing CHW infection, especially in microfilaria-negative dogs or dogs with low worm burdens. Recently, polymerase chain reaction (PCR) technology has been used for the detection and identification of specific microfilaria in peripheral blood samples.
• Amplification of DNA from circulating microfilaria in a multiplex PCR assay gave reliable positive results for samples containing as few as 4 microfilaria per mL and distinguished D immitis infections from D repens infections.10
• Another multiplex PCR assay was used to distinguish between 6 different species of microfilariae in dogs and resolve discrepancies in mor-
phologic diagnosis by 2 diagnostic laboratories. Molecular speciation identified microfilaria from several samples as *D immitis*, differing from an original morphologic diagnosis as *Acanthocheilonema (Dipetalonema) reconditum*.

Currently, the clinical relevance of PCR testing for CHW is low because it is not available through large commercial laboratories.

**Imaging**

Ultrasound and radiology can be useful tools for diagnosing and staging CHW disease. Radiographic findings in a heartworm positive dog can be variable due to several factors such as:

- Length of infection and corresponding disease progression
- Size of the population of worms present
- Worm location in the cardiopulmonary system

In an animal with recent CHW infection, there may be no radiographic changes present; however, as the duration of infection increases, common changes include (Figure 3):

- Right ventricular enlargement
- Pulmonary vasculature dilation
- Parenchymal lung infiltrates.

In dogs with severe disease, the pulmonary arteries can appear tortuous or have nodular opacities. Hepatomegaly and pleural effusion may also be seen radiographically if the progression of the CHW infection leads to right-sided heart failure.

On ultrasound, adult worms can appear as parallel hyperechoic lines present in the pulmonary artery and occasionally in the right ventricle (Figure 4). Echocardiography can also be a useful tool in detecting the extent of right heart enlargement and functional impairment due to CHW infection. The presence of worms in the orifice of the tricuspid valve in dogs with hemoglobinuria is indicative of caval syndrome.

**EVALUATION OF CHW ANTIGEN TESTS**

Given that in-clinic antigen assays are now the primary method for detecting CHW infection in dogs, it is important to know how well current
tests are performing. In the past 10 years, some tests have been reformulated and new tests have been introduced.

We evaluated the performance of 4 immunoassays currently available for point-of-care diagnosis of CHW infection. Samples from a total of 114 dogs were tested, including 51 negative and 10 positive ethylenediaminetetraacetic acid (EDTA) whole blood samples, and 15 negative and 37 positive serum or plasma samples (Table 1).

Test Results
Tests were designated A, B, C, or D. Concordance among the tests was excellent.

- The only discordant test was a weak positive on Test A that was negative on all other tests. The sample, which was whole blood from a healthy, low-risk dog, was rerun on all tests with the same results using both whole blood and plasma.
- None of the 114 samples run on Tests A, C, and D were invalid; 2 samples run on Test B did not develop a positive control line and were declared invalid. The same samples were repeated successfully on additional Test B units.

The ability of the CHW tests to correctly identify CHW diseased dogs (sensitivity) and CHW disease-free dogs (specificity) was determined using the described criteria for identification of diseased and nondiseased samples (Table 2). Tests B, C, and D each demonstrated 100% sensitivity and 100% specificity, while Test A demonstrated 100% sensitivity and 98.5% specificity.

### Table 1. Overview of Samples Used in This Study

<table>
<thead>
<tr>
<th>No. of Samples</th>
<th>Sample Source</th>
<th>Sample Type</th>
<th>Classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>51</td>
<td>CHW screening clinic (low risk)*</td>
<td>Whole blood</td>
<td>Negative</td>
</tr>
<tr>
<td>9</td>
<td>Annual CHW screening (low risk)*</td>
<td>Plasma</td>
<td>Negative</td>
</tr>
<tr>
<td>1</td>
<td>Laboratory (uninfected)§</td>
<td>Whole blood</td>
<td>Negative</td>
</tr>
<tr>
<td>6</td>
<td>Laboratory (uninfected)§</td>
<td>Serum/plasma</td>
<td>Negative</td>
</tr>
<tr>
<td>10</td>
<td>Laboratory (experimental infection)§</td>
<td>Whole blood</td>
<td>Positive</td>
</tr>
<tr>
<td>37</td>
<td>Laboratory (experimental infection)§</td>
<td>Serum/plasma</td>
<td>Positive</td>
</tr>
</tbody>
</table>

* Samples were collected from dogs participating in CHW screening or outpatient clinics at the College of Veterinary Medicine, Western University of Health Sciences. These animals were pets of veterinary faculty, staff, and students living in an area of Southern California with low prevalence of CHW infection. Most dogs (38/51 whole blood samples), including all dogs with history of living or travelling outside of California, were on a monthly CHW preventive program.

§ Samples were obtained from a commercial laboratory. These samples were collected from dogs that had been experimentally infected by IV transplantation with adult heartworms 4.5 to 12 months before sampling or inoculation of L3 larvae 1 to 3 years before sampling. Uninfected control dogs were also sampled.

### Table 2. Sensitivity & Specificity of CHW Tests

<table>
<thead>
<tr>
<th>Test</th>
<th>Test Name (Type; Manufacturer)</th>
<th>Sensitivity (No. positive/No. diseased)</th>
<th>Specificity (No. negative/No. disease-free)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>VetScan Canine Heartworm Rapid Test (ICT; abaxis.com)</td>
<td>100% (47/47)</td>
<td>98.5% (66/67)</td>
</tr>
<tr>
<td>B</td>
<td>Solo Step CH (ICT; heska.com)</td>
<td>100% (47/47)</td>
<td>100% (67/67)</td>
</tr>
<tr>
<td>C</td>
<td>Canine Heartworm Test (ICT; safepath.com)</td>
<td>100% (47/47)</td>
<td>100% (67/67)</td>
</tr>
<tr>
<td>D</td>
<td>SNAP Canine Heartworm Antigen Test Kit (ELISA; idexx.com)</td>
<td>100% (47/47)</td>
<td>100% (67/67)</td>
</tr>
</tbody>
</table>

ELISA = enzyme-linked immunosorbent assay; ICT = immunochromatographic test

All assays were run and scored by a single evaluator (Barr) according to the manufacturer’s directions for use. The evaluator was blinded to status of serum and plasma samples, but only partially blinded to status of whole blood samples due to awareness that most of these samples came from a low-risk population.
Potential Study Bias
Some potential for bias existed in the performance of diagnostic tests in this study.

- Heartworm-infected and noninfected animals were selected from distinctly different populations rather than from a spectrum of diseased and nondiseased subjects.14
- CHW-infected animals were all artificially infected. Of these 47 dogs, we received information on the infection dose for 25 dogs, with necropsy data available on 6 dogs.
- The worm burdens in the artificially infected dogs were similar to the mean number of worms reported for naturally infected dogs in Michigan, higher than those reported for Maine, and lower than those reported for Florida.8,15 However, marginally infected animals (worm burden of < 2 adult females), which can represent the largest numbers of false negative animals, were underrepresented and, therefore, our reported sensitivity values may be artificially elevated.

On the other hand, reported specificity values may be artificially elevated due to incorporation bias within the CHW negative group (see Standards for Heartworm Testing).14 The accepted gold standard is absence of adult worms at necropsy, but our disease-free population was defined by alternative criteria that included previous negative CHW antigen test results. This can lead to significant bias and artificial elevation of test specificity.

Minimizing Bias
The likelihood of bias was minimized through selection of traits that reduced the likelihood of these subjects to be infected including:

- Disease-free clinical presentation (all dogs)
- Residence in a low-risk area (all dogs)
- History of administration of CHW preventative (most dogs)
- Previous negative test results (multiple times in some dogs).

However, because the previous negative test results were determined by 1 of the 4 test kits (Test D) utilized in this study, the potential for bias, although small, is still present.

CONCLUSION
In most cases, diagnosis of CHW infection is relatively straightforward and easily accomplished through in-clinic testing. However, an understanding of the heartworm life cycle and recognition of the limitations of current diagnostic procedures are critical to successful diagnosis.

Two major limitations of most CHW tests are:

- Inability to detect infection during the long prepatent period
- Decreased sensitivity of detection when very few or no female worms are present.

Commercial CHW antigen tests have high sensitivity and specificity but performance may be compromised when these limitations are encountered.

Acknowledgements
We gratefully acknowledge veterinary technicians Kathryn Cresco, Eva Jaeger, and Kimberly Holt for their help with the CHW screening clinic. We also thank Dr. Brenda Knowlton and student representative Bridget Morton of Pfizer Animal Health for helping plan and organize the clinic.

CHW = canine heartworm;
EDTA = ethylenediaminetetraacetic acid;
ELISA = enzyme-linked immunosorbent assay;
ICT = immunochromatographic test;
PCR = polymerase chain reaction

References
Margaret C. Barr, DVM, PhD, is a professor of virology and immunology and serves as a coleader and facilitator for the Veterinary Basic and Clinical Sciences problem-based learning (PBL) course at the Western University of Health Sciences College of Veterinary Medicine. Dr. Barr is also the project director for the Snow Leopard Functional Genomics Initiative and serves on the board of directors for Pegasus Rising, an organization that provides equine-assisted therapy to veterans. Her interests include translational research on diagnostic assays for infectious diseases, nontraditional methods of immunization, emerging and reemerging infectious diseases, and host immune responses to infectious diseases. Current research projects include investigation of the molecular epidemiology of canine parvovirus and rickettsial agents in southern California. Dr. Barr received her DVM from Auburn University and her PhD from Cornell University.

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Peggy Schmidt, DVM, MS, Diplomate ACVPM, is an associate professor of population health and epidemiology at Western University of Health Sciences College of Veterinary Medicine. Dr. Schmidt serves as director of College Outcomes Assessment and coordinator of the WesternU DVM/MPH dual degree program. Her areas of research interest include evidence-based veterinary medicine, veterinary public health, infectious diseases, and veterinary education. Dr. Schmidt received her DVM from University of Minnesota. After working in a mixed-animal practice in western Minnesota, she joined Iowa State University College of Veterinary Medicine as an instructor while pursuing an MS degree in veterinary preventive medicine.

Frank Bossong, DVM, is an assistant professor in shelter medicine at Western University of Health Sciences College of Veterinary Medicine. He is also the head clinician for the Veterinary Ambulatory Community Service (VACS), a second year problem-based learning facilitator, and a course leader for the third-year internal medicine course. Dr. Bossong received his DVM from University of Georgia. After working as an associate for 4 years at an AAHA-accredited hospital in Pasadena, California, Dr. Bossong pursued his interest in shelter medicine. He established an on-site veterinary clinic, medical policies and procedures, and a fourth-year veterinary rotation at the San Gabriel Valley Humane Society, where he currently serves on the board of directors.

Gary R. Johnston, DVM, MS, Diplomate ACVR, is a professor of radiology at Western University of Health Sciences College of Veterinary Medicine and teaches all levels of the curriculum, facilitates problem-based learning, and participates in clinical research. Dr. Johnston received his DVM from Washington State University and then completed a rotating small animal medicine internship, MS, and residency in radiology at University of Minnesota. He remained at the University of Minnesota as a radiologist, progressing from assistant professor to full professor. Dr. Johnston spent 3 years at Washington State University College of Veterinary Medicine before joining the WesternU faculty.