Canine heartworm testing in Canada: Are we being effective?

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Abstract — Mass testing of dogs in Canada for the presence of *Dirofilaria immitis* has been ongoing since 1977. Since that time, there have also been changes in the diagnostic tests available to detect the presence of heartworm and changes in the therapy for heartworm, which necessitate a reevaluation of heartworm screening as currently practised in Canada. The principles of evidence-based medicine were used to determine the prevalence of heartworm infection in various dog populations, and the effectiveness of screening these populations. The annual surveys of heartworm testing have shown that Canada is a low prevalence area (0.16%), with most of the test-positive dogs located in southern Ontario (0.19%), southern Manitoba (0.18%), southern Quebec (0.09%), and the southern Okanagan Valley (0.04%). Foci of higher prevalence are found within these 4 main geographic areas. Furthermore, the prevalence of heartworm infection is higher in the population of dogs not on preventative medication (0.62%), when compared to the population of dogs on preventative medication (0.04%). The evidence indicates that a heartworm diagnostic test applied to an asymptomatic dog on preventative medication contributes little information regarding the heartworm infection status of that dog. However, testing of a dog characterized as being high risk will provide clinically useful information. Recommendations regarding the testing of dogs for heartworm in Canada are derived on the basis of available evidence.

Résumé — Recherche des vers du cœur chez le chien au Canada : sommes-nous efficace? La recherche systématique de *Dirofilaria immitis* chez les chiens au Canada est en cours depuis 1977. Depuis ce temps, il s'est produit des changements tant au niveau des méthodes de diagnostic disponible pour détecter la présence du ver du cœur qu'au niveau de la thérapie, ce qui nécessite une réévaluation du dépistage tel que pratiqué présentement au Canada. Une médecine basée sur l'observation a servi à déterminer la prévalence des infections par le ver du cœur chez différentes populations de chiens ainsi que l'efficacité du dépistage dans ces mêmes populations. Les enquêtes annuelles sur la recherche du ver du cœur ont montré que le Canada est un territoire de faible prévalence (0,16 %) avec concentrations des tests positifs dans le sud de l'Ontario (0,19 %), le sud du Manitoba (0,18 %), le sud du Québec (0,09 %) et le sud de la vallée de l'Okanagan (0,04 %). Des foyers de plus forte prévalence se retrouvent dans ces 4 régions géographiques principales. De plus, la prévalence des infections par le ver du cœur est plus élevée chez les populations de chiens ne recevant pas de traitement préventif (0,62 %) par rapport aux populations de chiens ne recevant un tel traitement (0,04 %). Les données indiquent qu'un test de diagnostic du ver du cœur effectué chez un chien symptomatique recevant un traitement préventif n'apporte que peu d'information sur le bilan de l'infection pour le ver du cœur chez ce chien. Cependant, le test réalisé chez un chien considéré à haut risque apportera des renseignements cliniques utiles. Les recommandations concernant la recherche du ver du cœur chez le chien sont formulées à partir des données disponibles.

(Traduit par docteur André Blouin)

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Introduction

The use of *Dirofilaria immitis,* commonly referred to as "heartworm," detection tests as part of an overall preventative medicine package or, less commonly, as part of a diagnostic work-up, is widespread in Canada. However, the extensive use of these tests has raised important questions with respect to the merits of annual screening, as well as test selection and interpretation, especially when used on asymptomatic dogs. These issues stimulated the authors to initiate a review of the available information on heartworm testing, as it pertains to the situation in Canada. An evidence-based approach was used, looking at what is publically documented about the prevalence of heartworm in Canada and about the characteristics of the tests. The information is summarized to provide practitioners with a better understanding of the interpretation of test results and the effectiveness of "annual screening" for heartworm in Canada. Recommendations for heartworm testing and treatment options are given at the end of the text.

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Reprints will not be available.

Materials and methods

Estimates of the prevalence of heartworm infection in Canada were ascertained from the reports on the annual surveys carried out over the last 23 years by Dr. J.O.D. Slocombe and associates (1–17).

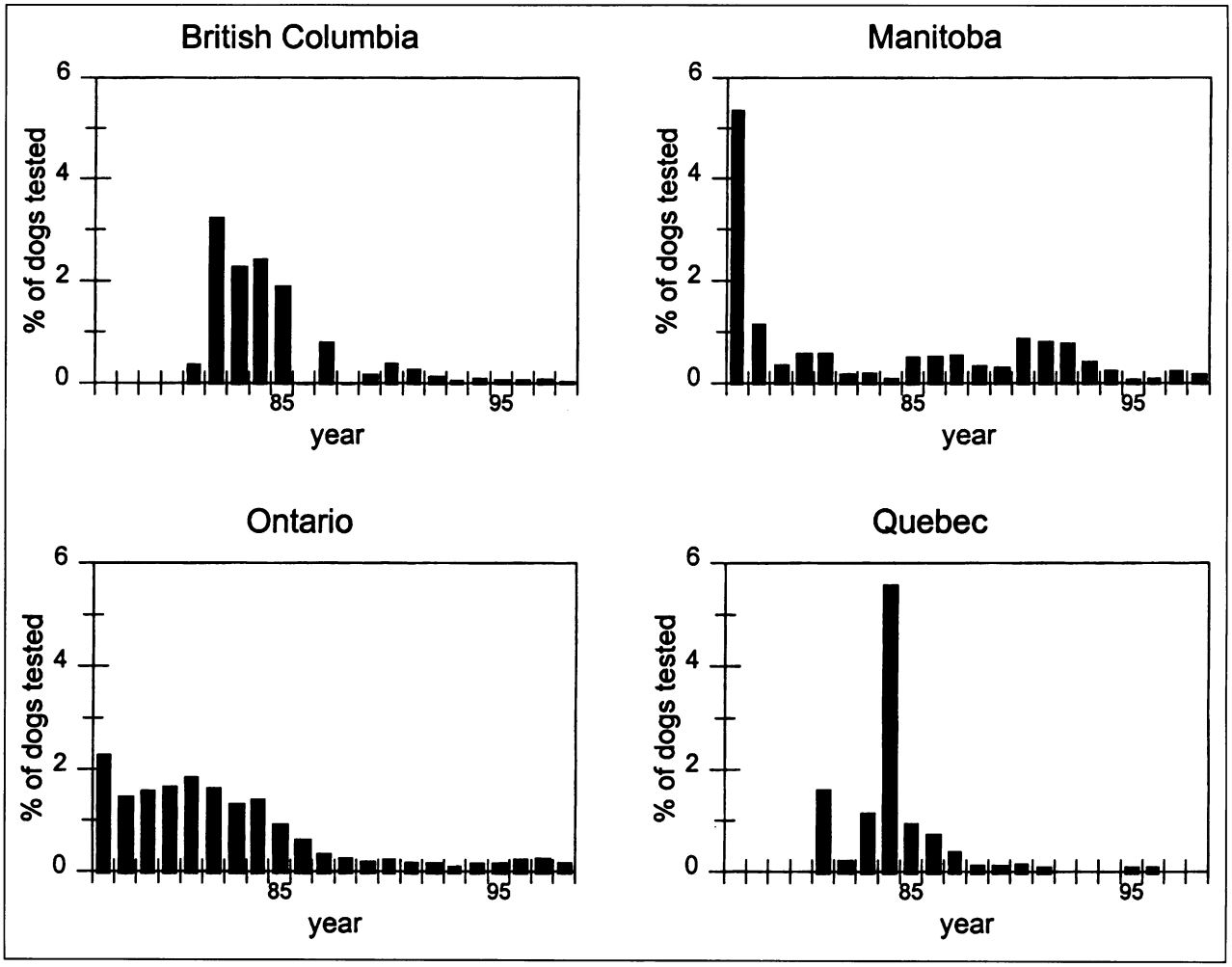

Figure 1. The prevalence of heartworm infection in dogs tested in the 4 endemic foci in Canada from 1977 to 1998.

The published literature was searched for English-language articles on heartworm tests by using Medline (18), CAB (19), and Current Contents (20). The databases were searched from 1980 to 1999, using the search sequences "heartworm and dogs," "dirofilariasis and canine," "heartworm antigen," and "diagnostic test and heartworm." All listed references in the articles found in the database searches were also scrutinized, as were Web sites of companies offering heartworm detection kits or services. Eligibility of articles for critical review was based on the use of a "gold standard" for detection of heartworm, namely necropsy, and the use of "blinding" in the study design, where the person performing the heartworm test was unaware of the necropsy results. Results were reported only for heartworm test kits available to Canadian practitioners (as of 1998).

Results and discussion

Heartworm prevalence in Canada

Infection with *D. immitis* was first considered to be endemic to Canada in the 1970s. Since then, the annual heartworm testing done by practitioners, as reported in the annual surveys conducted by Dr. J.O.D. Slocombe and associates (1–17), have provided estimates of the prevalence in Canada and its secular trend. Generally, the prevalence of heartworm infection is low and has apparently decreased from 1.31% in 1978 to an overall prevalence of 0.16% in 1998 (16). However, these estimates may be biased, because the surveys included only those dogs that visited a veterinarian in a given year and whose owners agreed to testing. In addition, the response rates for the surveys were lower than desired, ranging from 50% to 60%, and only 20% to 55% of the results were obtained from clinic records. As well, dogs with a history of travel into endemic areas outside Canada were included, and a variety of diagnostic tests were used. The use of less-than-perfect tests and the widespread use of preventative medication further complicated assessment of the actual prevalence. Nevertheless, it appears that the prevalence of heartworm infection is geographically focal in nature.

Initially, 2 foci of heartworm infection were recognized in Canada, one in southern Ontario and one in southern Manitoba (1–7). In 1984, a 3rd focus was identified in southern Quebec, around Montreal (8). In 1991, a cluster of cases of clinical and subclinical heartworm was reported in the southern Okanagan Valley of British Columbia (21). These 4 regional foci have persisted (Figure 1). The apparent prevalence in all dogs tested in the 4 endemic foci of Canada in 1998 was 0.04% (1/2734) in the Okanagan valley; 0.18% (23/13 111) in

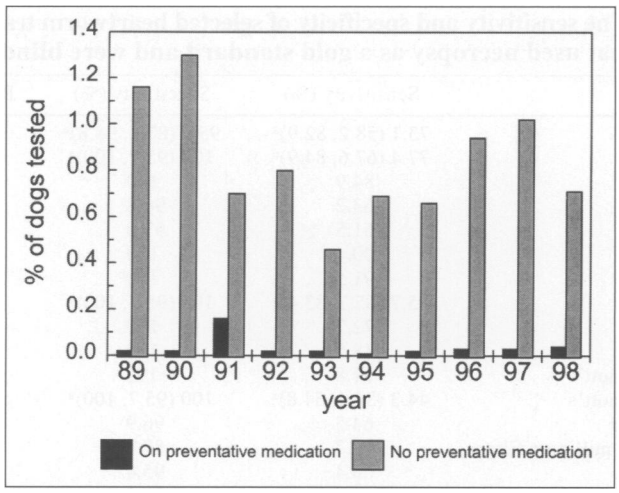

Figure 2. The preventative medication-specific prevalence of heartworm infection in dogs tested in Ontario from 1989 to 1998.

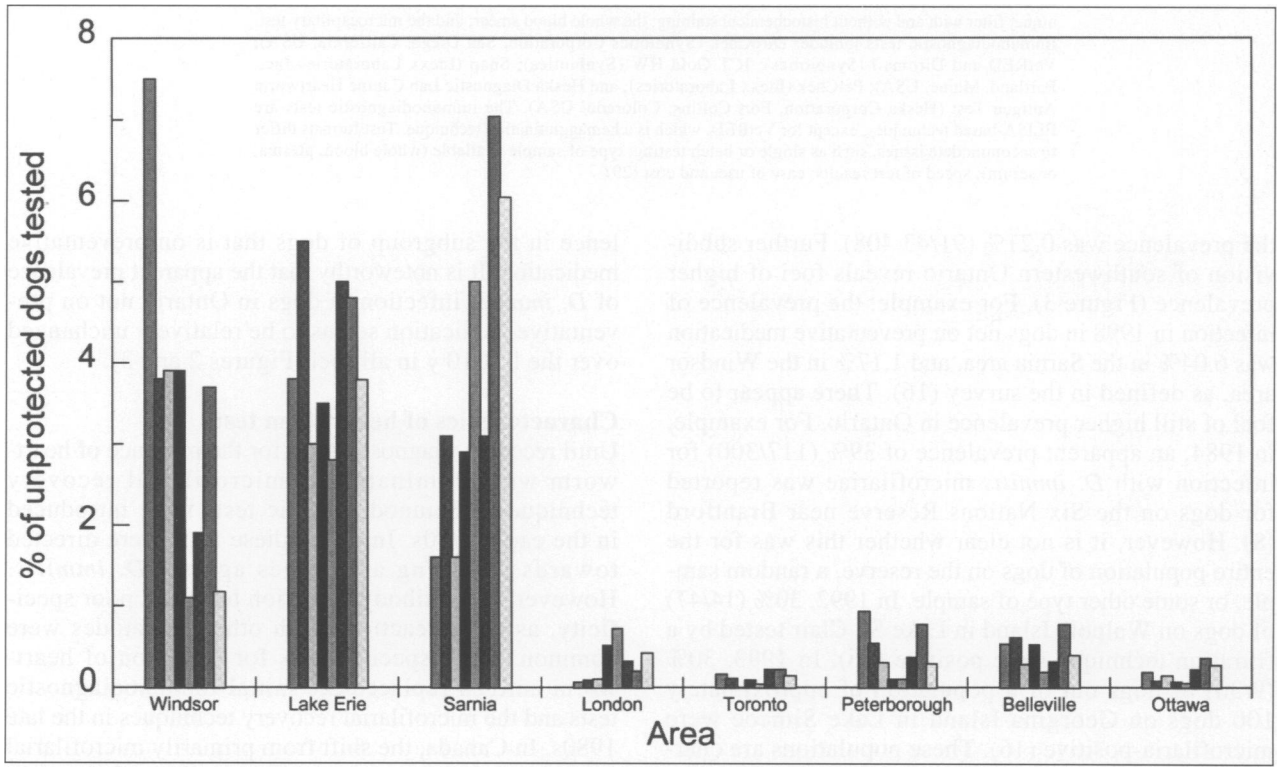

Figure 3. The prevalence of heartworm infection in unprotected dogs in Ontario from 1991 to 1998. The areas are a composite of practices within or surrounding the descriptor, according to the watershed divisions, as defined by Slocombe (16).

southern Manitoba; 0.19% (537/282 540) in southern Ontario; and 0.09% (89/95 151) in southern Quebec (1996 data) (16,17). Within these regional foci, the majority of test-positive cases have tended to cluster in the same practice areas year after year. For example, in Ontario, 75% to 80% of the test-positive cases come from practices in southwestern Ontario, defined as south of a line drawn from Sarnia, along Hwy 402 to London, along Hwy 401 past Woodstock, and then along Hwy 403 to Hamilton. The apparent prevalence in all dogs in this geographic area tested in 1998 was 0.46% (424/91 592), while the apparent prevalence in the rest of Ontario was 0.06% (126/202 025).

Prevalence estimates also differ between the population of dogs on preventative medication and the population not on preventative medication. In Canada, the prevalence of heartworm infection in dogs on medication and tested in 1998 was 0.04% (97/247 011), while the prevalence was 0.62% (481/77 846) for dogs not on medication. In Ontario, the overall prevalence in dogs tested in 1998 that were or were not on preventative medication was 0.04% (84/226 414) and 0.74% (462/62 875), respectively (Figure 2). In southwestern Ontario, as defined above, the prevalence of infection in dogs tested in 1998 and not on preventative medication was 1.91% (371/19 467), whereas in the rest of Ontario,

Table 1. The sensitivity and specificity of selected heartworm tests from studies that used necropsy as a gold standard and were blinded

Antigen test	Sensitivity (%)	Specificity (%)	Reference
DiroCHEK	73.1 (58.2, 82.9)[a]	95.9 (87.1, 98.8)[a]	22
DiroCHEK	77.4 (67.6, 84.9)[a]	100 (95.7, 100)[a]	26
DiroCHEK	84.9	100	27
VetRED	63.2	96.8	25
VetRED	61.5	97.0	23
VetRED	90.4	100	23
ICT Gold	91.5[b]	100[b]	24
PetChek	75.7 (65.7, 83.4)[a]	100 (95.7, 100)[a]	26
PetChek	72.7	100	27
Snap	48.5	100	27
Modified Knott's	81.8	100	27
Modified Knott's	44.3 (34.4, 54.8)[a]	100 (95.7, 100)[a]	26
Direct smear	64.5	96.9	28[c]
Filter (5 μm millipore filter)	72.7	87.7	28[c]
Knott's	66.4	95.4	28[c]
Capillary tube	60.0	96.9	28[c]

[a]95% confidence interval
[b]Gold standard and blinded data were available for 1 of 4 sites only
[c]Study not blinded
In Canada, the currently available microfilarial recovery tests include: the modified Knott's technique; filter with and without histochemical staining; the whole blood smear; and the microcapillary test. Immunodiagnostic tests include: DiroChek (Synbiotics Corporation, San Diego, California, USA); VetRED and Diromail (Synbiotics); ICT Gold HW (Synbiotics); Snap (Idexx Laboratories Inc., Portland, Maine, USA); PetChek (Idexx Laboratories); and Heska Diagnostic Lab Canine Heartworm Antigen Test (Heska Corporation, Fort Collins, Colorado, USA). The immunodiagnostic tests are ELISA-based techniques, except for VetRED, which is a hemagglutination technique. Test formats differ to accommodate issues, such as single or batch testing; type of sample available (whole blood, plasma, or serum); speed of test results; ease of use; and cost (29)

the prevalence was 0.21% (91/43 408). Further subdivision of southwestern Ontario reveals foci of higher prevalence (Figure 3). For example, the prevalence of infection in 1998 in dogs not on preventative medication was 6.04% in the Sarnia area, and 1.17% in the Windsor area, as defined in the survey (16). There appear to be foci of still higher prevalence in Ontario. For example, in 1984, an apparent prevalence of 39% (117/300) for infection with *D. immitis* microfilariae was reported for dogs on the Six Nations Reserve near Brantford (8). However, it is not clear whether this was for the entire population of dogs on the reserve, a random sample, or some other type of sample. In 1992, 30% (14/47) of dogs on Walpole Island in Lake St. Clair tested by a filtration technique were positive (16). In 1993, 30% (9/30) of dogs out of a population of approximately 100 dogs on Georgina Island in Lake Simcoe were microfilaria-positive (16). These populations are characterized by a large number of outdoor dogs in close proximity to each other. There appears to be no significant spread from these foci, since dogs situated in areas surrounding the foci do not show an elevated prevalence of heartworm infection beyond the expected background level.

Time trends

Although the heartworm foci have persisted, the overall apparent prevalence of heartworm in Canada has generally declined since 1984 (Figure 1). A large proportion of this decline is probably because most dogs tested are already on preventative medication. The number of dogs tested in Ontario in 1998 that were on preventative medication was 201 089 (76.8% of all tested dogs) (16). The overall estimated prevalence in tested dogs, therefore, is heavily influenced by the prevalence in the subgroup of dogs that is on preventative medication. It is noteworthy that the apparent prevalence of *D. immitis* infection in dogs in Ontario not on preventative medication seems to be relatively unchanged over the last 10 y in all foci (Figures 2 and 3).

Characteristics of heartworm tests

Until recently, diagnostic tests for the presence of heartworm were dominated by microfilarial recovery techniques. Immunodiagnostic tests were introduced in the early 1980s. Initially, these tests were directed towards detecting antibodies against *D. immitis*. However, the antibody detection tests had poor specificity, as cross-reactions with other nematodes were common. More specific tests for detection of heartworm antigen replaced the initial immunodiagnostic tests and the microfilarial recovery techniques in the late 1980s. In Canada, the shift from primarily microfilarial recovery techniques to antigen detection techniques in dogs began in 1992, and in 1998, accounted for approximately 95% of the testing in Ontario, 97% of the testing in Manitoba, 98% of the testing in British Columbia, and 25% of the testing in Quebec (1996 data) (14–17).

The literature search yielded 6 eligible articles assessing heartworm antigen tests (22–27). Since no blinded study was available for the microfilarial recovery techniques, an unblinded study was used to assess the characteristics of these techniques (28).

Sensitivity and specificity

In general, there are very few valid reports on the characteristics of heartworm diagnostic tests. In addition, the available data may be biased, as the study subjects were selected from populations with a suspected high prevalence of infection and were not representative of the

population at large in the study area, let alone in Canada, a low prevalence area. As well, in Canada, dogs are typically screened 6–8 mo after the predicted last possible transmission date (15,16) and the timing of blood sampling and necropsy postinfection was not specified in any report. Finally, in some studies, the end-point of the tests was not clear. The reported sensitivities (the proportion of dogs with heartworm that test positive) and specificities (the proportion of dogs without heartworm that test negative) of several antigen tests and microfilarial recovery techniques are presented in Table 1.

The sensitivity of heartworm antigen tests depends on the worm burden, and the sex and age of the parasites. In a study with 61 teaching dogs at Oklahoma State University (26), the sensitivity of these tests was found to decrease substantially with worm burdens of less than 5. For example, with DiroChek (Synbiotics Corporation, San Diego, California, USA), the sensitivity decreased from 100% in dogs with more than 10 worms, to 70% to 80% in dogs with less than 5 worms. The sensitivity of PetChek (Idexx Laboratories, Portland, Maine, USA) decreased from 100% in dogs with more than 10 worms, to 40% to 50% in dogs with less than 5 worms. Dzimianski, Tier, and McCall (33) found that the sensitivity of the tests increased substantially when the adult female worm burden increased to 3 or more worms of at least 7 mo of age. Infections less than 5 mo old and unisex male worm burdens were usually not detected. It should be noted that all of the test assessments reported above were derived from populations of dogs in which the heartworm burden was an average of 7 to 15 worms/dog. This burden may not apply in low prevalence areas, such as most of Canada. Unfortunately, no data are available for average worm burdens in dogs that have acquired heartworm infections in Canada.

The sensitivity of microfilarial recovery techniques is primarily determined by the occult rate in the study population. The occult rate is associated with prepatent infections, unisexual infections, drug-induced sterility of adults or death of microfilariae, and immune-mediated clearance of microfilariae (30). In Canada, the occult rate in dogs appears to range from 25% to 30% (16). It should, however, be noted that the sensitivity of microfilarial recovery techniques is also reduced by low blood microfilarial densities. Testing characteristics, such as apparatus leaks or occlusions, excess fluid removal from the edge of the coverslip, microfilariae sticking to the side of the centrifuge tube, and the analyzed volume of blood will also affect the sensitivity (31,32). The modified Knott's technique and the filtration techniques are preferred over other recovery techniques, as they examine a larger volume of blood (1.0 mL) and are, therefore, considered more sensitive.

The specificity of most of the antigen tests appears to be very high, although the cited specificities of 100% are likely a reflection of the low numbers of dogs tested. False-positive results can occur when adults have died less than 3 mo prior to testing, as antigen may take this long to dissipate (35); when the monoclonal antibody binds to antigens from other nematodes or to other agents in the sera (especially if hemolysis or lipolysis is evident); and when nonspecific binding to a solid surface occurs (36).

The specificity of the microfilarial recovery techniques is affected by confusion of *D. immitis* microfilariae with the microfilariae of other filarid species, plant fibers, or other debris, or when contamination of the test equipment and fluids has occurred. Differentiation among filarid species is generally much easier with the modified Knott's technique (length, width of body, shape of tail and head) than with the filtration and staining technique (shape of head only) (31,32). Specificity is also lowered when a microfilaremia persists after death of adult parasites, which can occur naturally or after adulticide treatment. Clinically, microfilaremias have been found to persist for 6 to 18 mo following treatment with thiacetarsamide (34), and presumably the same is possible after natural death of the adults. In the study conducted by Martini et al (28), 4.6% (8/175) of the tested population were described as microfilaremic with no adults.

For the reasons stated above, the listed sensitivities and specificities of the available tests for heartworm may not be valid for the Canadian dog population, or, at least, the population of dogs seen by Canadian veterinarians. However, given the evidence, as indicated above and in Table 1, worse-case estimates of the sensitivity and specificity of the heartworm antigen tests are 40% to 85%, and 99.3%, respectively. There is no indication of what the actual sensitivity of the antigen tests are in Canada, but it should be maximized if testing is conducted no earlier than 7 mo postinfection, or not before mid-May of the following year, given that October is the last possible month that infection can occur in Canada. The worse-case estimate of 99.3% [(62 875–462)/62 875] for the specificity of the antigen tests was calculated from the 1998 Ontario survey (16) by using test results in unprotected dogs and assuming all the positive tests were false. However, given the uncertainty of how well the antigen detection tests operate in low prevalence areas, a middle estimate, that is, an estimate half-way between the worse-case estimate and perfect specificity, can provide a basis for further calculations. Thus, if we assume an average, that is that 50% of the test-positive results are false, the calculated specificity for the antigen tests increases to 99.6% [(62 875-462/2)/62 875].

Worse-case estimates of the sensitivity and specificity of the Knott's and filtration techniques, in the Canadian context, are 75% and 98%, respectively, assuming an occult rate of 25% and calculating an estimate of the specificity from the 1981 survey data by assuming that all the test-positive results are false [(31 323–560)/31 323)] (5). Again, given the lack of performance evaluation of the microfilarial recovery techniques in low prevalence areas, if we assume that 50% of the test-positive results are false, the estimate of the specificity for the Knott's and filtration techniques increases to 99.1% [(31 323–560/2)/31 323].

Reliability

Another important feature of a diagnostic or screening test is its reliability or repeatability, that is, the ability of the test to give the same results upon repetition. Unreliability appears to be a problem with heartworm antigen tests, as many practitioners are concerned with discrepant results on retesting of dogs. Data on reliability

Table 2–1. Calculation of a heartworm test's operating characteristics

Screening test results	Heartworm infection status of the dog			
	Infected	Not infected	Totals	Predictive values
Positive	A	B	A + B	PPV = A/(A + B)
Negative	C	D	C + D	NPV = D/(C + D)
Totals	A + C	B + D	The total number of animals tested is n = A + B + C + D	
	Sensitivity A/(A + C)	Specificity D/(B + D)		

Pretest probability is the proportion of the population that is infected with heartworm. The pretest probability = (A + C)

A — the number of true-positive dogs; B — the number of false-positive dogs; C — the number of false-negative dogs; D — the number of true-negative dogs; PPV — positive predictive value, the proportion of test-positive animals that are infected; NPV — negative predictive value, the proportion of test-negative animals that are not infected

Sensitivity of the test is the proportion of heartworm-infected dogs that will test positive. The proportion that will test negative is known as the false negative rate

Specificity of the test is the proportion of heartworm-free dogs that will test negative. The proportion that will test positive is known as the false positive rate

Table 2-2. An example of the calculations to estimate the number of test-positive and test-negative animals and their likelihood of being infected with heartworm in your area. Assume your practice is in Toronto. You are presented with an asymptomatic dog that has never been on heartworm preventative medication and has never been outside Toronto. From the annual surveys (16), the pretest probability of heartworm infection in unprotected dogs in the Toronto area is 0.14%. Assume that this is the true pre-test probability of infection. The heartworm test you have in your clinic is PetChek® (sensitivity is 75%; specificity is 99.6%). To begin the calculations, for areas with low prevalence, results are much clearer if we assume 100 000 dogs from the area are tested. Multiply this by the pretest probability of infection to get the total number of infected dogs (100 000 × 0.0014). Obtain the total number of uninfected dogs by subtraction (100 000 − 140). Now multiply the sensitivity by the number of infected dogs (0.75 × 140) and the specificity by the number of uninfected dogs (0.996 × 99 860) to complete the A and D cells of the table. The values for the C and B cells of the table are obtained by subtraction from the column totals. By calculating the row totals, one can then determine the total number of test-positive and test-negative dogs. These totals then become the denominators for calculation of the PPV and NPV, respectively.

PetChek test results	Heartworm infection status of the dog			
	Infected	Not infected	Totals	Predictive values
Positive	A = 140 × 0.75 = 105	B = 99 860 − 99 461 = 399	A + B = 105 + 399 = 504	PPV = A/(A + B) = 105/504 = 0.208 (20.8%)
Negative	C = 140 − 105 = 35	D = 99 860 × 0.996 = 99 461	C + D = 35 + 99461 = 99 496	NPV = D/(C + D) = 99 461/99 496 = 1.00 (100%)
Totals	A + C = 100 000 × 0.0014 = 140	B + D = 100 000 − 140 = 99 860	The total number of animals tested n = 100 000	

are sparse but appear to indicate that there may be some cause for concern. In a study conducted by Hoover et al (27), discordant replicated test results for 8 different tests were reported to have eliminated dog samples from further analysis; the frequency with which this occurred was not discussed. In contrast, Courtney and Zeng (26) found that duplicate tests run on the same 224 serum samples with the same test kit were identical for both the DiroChek and PetChek test. However, in an unblinded study conducted by Matherne et al (37), the DiroChek test kit used on duplicate samples had a difference in interpretation of positive and negative between 2 different laboratories in 2% of samples. Part of the problem with all of the antigen tests appears to be associated with the intensity of the color development or the hemagglutination reaction; a faint color change or mild hemagglutination becomes a subjective interpretation, even with the use of positive and negative controls. In order to eliminate such subjectivity, spectrophotometric readings were made on replicate samples tested

with PetChek and DiroChek (38). Color intensity did vary between replicated samples. In addition, it was found that the readings for positive DiroChek samples continued to increase after the recommended incubation period, whereas the readings for positive PetChek results did not, likely due to the absence of a "stop" reagent that is included in the PetChek kit. Negative results did not vary in color intensity with either test.

Test interpretation

In order for practitioners to rationally interpret the results of a heartworm test, information on sensitivity and specificity of the test and the estimated pretest probability of infection in the dog have to be combined to obtain positive and negative predictive values. The predictive values aid the practitioner in making a decision about the infection status of a dog. The positive predictive value (PPV) is the probability that a test-positive animal is truly infected with *D. immitis*. The negative predictive value (NPV) is the probability that a test-negative animal is not infected with *D. immitis*. Table 2–1 shows how the predictive values are calculated, and Table 2–2 provides an example of the effectiveness, or utility, of testing a dog with a low pretest probability of heartworm infection with PetChek.

Estimates of the sensitivity and specificity for PetChek are 75%, reflecting the estimate in the published literature (26,27), and 99.6%, reflecting the estimate from the 1998 Ontario survey (described above), respectively. PetChek was chosen as an in-clinic test because of its "stop" reagent, which stops color development after addition, allowing a more accurate assessment of the result if the operator cannot read the test directly after the required incubation period. For the practitioner, the best estimate of the pretest probability of infection is derived from prevalence estimates in previous years. Major determinants of the pretest probability of infection in an individual dog are the use of preventative medication, the degree of compliance with the preventative program, and travel into a heartworm endemic area. These determinants can lead to an approximate 10-fold difference in pretest probability. For example, in a typical area outside the heartworm endemic zone in Ontario, the likely pretest probability of heartworm infection in dogs not on medication is 0.2%, whereas in dogs on medication, it is 0.02% (31/158 617) (16). In Table 3, we show the positive and negative predictive values for a heartworm antigen test at various pretest probabilities of infection, using the sensitivity and specificity estimates given above. Table 4 shows similar data for the Knott's or filtration tests, using the sensitivity and specificity estimates of 75%, reflecting the occult rate, and 99.1%, reflecting the estimate calculated from the 1981 survey, respectively.

At all pretest probabilities of heartworm infection, from 0.02% to 30.0%, a negative test result for all heartworm tests will effectively rule out heartworm infection. That is, one is 90% to 100% certain that the animal is truly not infected. In contrast, a positive test is a poor predictor of infection, except when the pretest probability of heartworm infection is relatively high. More specifically, if the pretest probability of heartworm infection is less than 5.0%, the PPV drops off rapidly, making the decision to treat too uncertain (see Table 3). Considering the expense of adulticide treatment and the potential side effects, with no guarantee of a complete cure, a practitioner should be at least 90% sure that an asymptomatic dog is truly infected before considering adulticide treatment. Thus, referring to Table 2–2, given that the low pretest probability of heartworm infection in unprotected, asymptomatic dogs in Toronto is 0.14% (16), one is 99.86% certain that the dog is not infected before the test is done. A positive result for a heartworm antigen test performed on such a dog has a predictive value of only 20.8%; only 1 in every 5 positive test results is likely to be a true positive. Therefore, a positive test result, in this situation, should not change the practitioner's opinion of the infection status before the test was done. It is only when the pretest probability of heartworm infection is at least 3% to 5% that a positive test result, by itself, should change the practitioner's opinion of the infection status of an unprotected, asymptomatic dog, as a PPV of 90% will be achieved. From the surveys, the pretest probability for heartworm infection in a previously test-negative dog on a consistent preventative medication program is 0.02% (16). Performing a heartworm antigen test on such a dog results in a PPV of only 3.6% (see Table 3). In this case, only 1 out of 28 test-positive dogs, with the above described characteristics, is likely to be truly infected, calling into question the rationale for an annual screening program of protected dogs.

High PPVs are likely to be achieved when high risk dogs are screened for heartworm. For example, if the dog

Table 3. The predictive values when an asymptomatic dog is tested with PetChek (sensitivity of 75% and specificity of 99.6%) at various pretest probabilities of infection with *Dirofilaria immitis*

Pretest probability	Positive predictive value	Negative predictive value
0.02%	3.6%	100.0%
0.2%	27.3%	100.0%
0.5%	48.5%	99.9%
1.0%	61.1%	99.8%
2.0%	79.3%	99.5%
5.0%	90.8%	98.7%
10.0%	95.4%	97.3%
30.0%	98.8%	90.3%

Table 4. The predictive values for microfilarial recovery techniques (Knott's or filtration) with a sensitivity of 75% and a specificity of 99.1% when performed on blood from asymptomatic dogs with various pretest probabilities of infection with *Dirofilaria immitis*

Pre-test probability	Positive predictive value	Negative predictive value
0.02%	1.6%	100.0%
0.2%	14.3%	100.0%
0.5%	29.5%	99.9%
1.0%	45.7%	99.8%
2.0%	63.0%	99.5%
5.0%	81.4%	98.7%
10.0%	90.3%	97.3%
30.0%	97.3%	90.2%

is symptomatic, with clinical signs and radiographic findings consistent with heartworm disease, and has resided in a heartworm endemic focus, the pretest probability of infection will likely be well above 10%. In this situation, a positive heartworm antigen test will confirm infection, as the PPV will be greater than 90%. For an unprotected, asymptomatic dog, the effectiveness of a screening program depends on the prevalence of heartworm infection in the area in which the dog resides during the transmission season, whether in Canada or abroad; the likely sensitivity and specificity of the screening test; and the level of certainty (the PPV) with which the clinician is comfortable.

Conclusions

Based on our review, we make the following recommendations for heartworm testing in Canada:

(1) Screening for heartworm infection should be an informed client-based decision, because the prevalence of infection is low and focal in nature.

(2) Screening for heartworm in low prevalence areas is only effective if the dog is in a high risk group. In Canada, a dog is in such a group if (a) the historical prevalence of heartworm infection in dogs in the area is greater than 3% or (b) the dog has a history of travel into high prevalence areas, and (c) there is a lack of use of, or compliance with, preventative medication. Thus, the travel history of the dog, coupled with the history of the use of preventative medication, remains an important part of the clinical examination. Current evidence suggests that few unmedicated dogs from outside southwestern Ontario should be considered as belonging to a high risk population of dogs.

(3) Preventative medication is efficacious (39,40), and testing of dogs on preventative medication is generally not clinically informative. A dog that has tested negative in the past and has been on heartworm preventative medication consistently during the parasite transmission season, whether in Canada or abroad, will have a pretest probability of being infected of virtually 0%. In the surveys in Ontario, the "apparent" prevalence of infection in dogs on preventative medication is 0.02%. However, this prevalence includes dogs that tested positive, and were not treated with an adulticide but put on preventative medication, as well as dogs with compliance failures and false-positive results. At a prevalence of 0.02%, the PPV of the antigen tests for heartworm infection is only 3.6%. Thus, for dogs on a consistent preventative medication program, only 1 out of every 28 positive antigen tests is likely to be a true positive. Moreover, of these 28 test-positive dogs, one cannot conclude which is the truly positive dog without obtaining information on the dog's history and clinical signs, or through the application of other diagnostic tests. Thus for dogs on preventative medication, especially if there is good owner compliance, there is no justification, at present, for carrying out annual heartworm screening.

(4) If retesting is being considered for a dog that is positive on a heartworm antigen test, the issue of imperfect repeatability should be taken into account. It should be recognized that there is incomplete agreement among antigen tests (26–28) and between antigen tests and microfilarial recovery techniques (consider the occult infection). If a dog is asymptomatic and not on preventative medication, retesting could probably wait for 3 mo or longer to allow for further parasite development and, potentially, an increased level of antigenemia or microfilaremia.

(5) Heartworm antigen testing is preferred in symptomatic and high-risk dogs, because it has a higher sensitivity and specificity than do microfilarial recovery techniques. On the other hand, microfilarial recovery testing is recommended if the dog is going to be put on daily preventative treatment with diethylcarbamazine (DEC), as a severe, potentially life-threatening condition may occur following treatment with DEC, due to massive death of microfilariae (41). Microfilarial recovery techniques should also be considered in symptomatic or high-risk dogs prior to beginning monthly milbemycin treatment, as this drug has the potential of causing a similar, but usually much less severe, reaction because of its rapid microfilaricidal activity at the preventative dose (42). There is less concern over similar side effects with ivermectin, as the monthly preventative dose is not considered microfilaricidal (43).

(6) Rarely should a veterinarian make or accept a recommendation for euthanasia of a dog in Canada testing positive for heartworm. Heartworm infection does not invariably lead to disease and death. In fact, it is generally accepted that low heartworm burdens (< 20 worms) are rarely of clinical significance (41), although such dogs should be monitored more closely. It would appear from the surveys that, in Canada, more dogs are euthanized because they tested positive than would ever become symptomatic or die from heartworm infection. Alternative treatment options for the asymptomatic dog testing positive for heartworm are as follows (44): adulticide treatment(s) only (to attempt a cure); adulticide treatment(s) followed by a microfilaricide (to attempt a cure and eliminate microfilariae); preventative medication only on a monthly basis for longer than 9 mo (to prevent further infection and induce an irreversible amicrofilaremic state) (45); or no treatment at all. If the dog is microfilaremic and the owner refuses adulticide and preventative medication, recommendations regarding the risk of heartworm transmission that this dog poses to itself, and other dogs in the area, can also be given. The risk, depends on the probability of mosquito contact with the infected animal, the probability of larval development within those mosquitoes to the infective stage, and the probability of those mosquitoes again contacting canine hosts. Information on the seasonality of heartworm transmission in Canada (46) and elsewhere (47), and on mosquito life cycles and behavior (48–49), can be used to inform clients about the likelihood of contact between their dog and heartworm-infected mosquitoes. CVJ

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