

Article

The low seroprevalence of tick-transmitted agents of disease in dogs from southern Ontario and Quebec

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Abstract – Infectious diseases caused by pathogens transmitted by ticks and other insect vectors are an important cause of morbidity and mortality in both dogs and humans throughout North America. The purpose of this study was to determine the seroprevalence of selected vector-transmitted pathogens in southern Ontario and Quebec. Samples submitted to the Vector Borne Disease Diagnostic Laboratory (VBDDL) at the North Carolina State University College of Veterinary Medicine were evaluated for antibodies to *Ehrlichia canis*, *Anaplasma phagocytophilum*, *Babesia canis*, *Bartonella henselae*, *Borrelia burgdorferi*, *Bartonella vinsonii* subspecies *berkhoffii*, and *Rickettsia rickettsii*. Information regarding breed and the city or province from which the sample originated was recorded; however, travel history was unknown for the majority of dogs. Overall seroprevalence to these tick-borne pathogens in southern Ontario and Quebec is low compared with most regions of the United States, suggesting that veterinarians in this region of Canada should pursue diagnostic evidence of infection in dogs with a travel history or prior residence in areas endemic for exposure to tick-borne infections.

Résumé – Faible séroprévalence de certains agents infectieux transmis par les tiques chez les chiens du sud de l'Ontario et du Québec. Les maladies infectieuses causées par des pathogènes transmis par les tiques et autres insectes vecteurs sont une importante source de morbidité et de mortalité à la fois chez le chien et l'homme dans toute l'Amérique du Nord. Le but de cette étude était de déterminer la séroprévalence de pathogènes particuliers, transmis par vecteurs, dans le sud de l'Ontario et du Québec. Les échantillons ont été transmis au Vector Borne Disease Diagnostic Laboratory (VBDDL) au North Carolina State University College of Veterinary Medicine pour être soumis à une évaluation des anticorps contre *Ehrlichia canis*, *Anaplasma phagocytophilum*, *Babesia canis*, *Bartonella henselae*, *Borrelia burgdorferi*, *Bartonella vinsonii* sous-espèce *berkhoffii* et *Rickettsia rickettsii*. Les renseignements concernant les races et les villes ou provinces d'origine des échantillons ont été notés mais l'historique du déplacement des chiens était inconnu dans la majorité des cas. La séroprévalence globale de ces pathogènes transmis par les tiques dans le sud de l'Ontario et du Québec est faible comparé à celle de la majorité des régions des États-Unis. Les vétérinaires de ces régions du Canada devraient être sensibilisés aux signes diagnostiques d'infection chez les chiens ayant voyagé ou résidé dans des endroits où l'exposition aux infections transmises par les tiques est endémique.

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Introduction

Infectious diseases caused by pathogens transmitted by ticks and other vectors are an important cause of morbidity

and mortality in both humans and dogs throughout North America. Notable etiologic agents in veterinary medicine include *Anaplasma phagocytophilum*, *Babesia canis*, *Bartonella*, *Borrelia burgdorferi*, *Ehrlichia canis*, and *Rickettsia rickettsii*. While numerous studies have described the seroprevalence and geographic distribution of these vector-borne organisms throughout the United States (1–7), little information is available regarding the seroprevalence in Canada. Knowledge of the seroprevalence, combined with the known distribution of vector ticks, will aid the veterinarian in selecting appropriate diagnostic tests and optimal treatment regimens, while awaiting test results. Additionally, definitive documentation of vector-borne infections in dogs can provide important sentinel information for the potential of human infection in a defined geographic location, which has important public health implications (8,9).

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The seroprevalence of many tick-transmitted pathogens is directly correlated to the geographic distribution of the primary vectors that transmit the organism (1,10,11). For instance, the seroprevalence of *B. burgdorferi*, the etiologic agent of Lyme borreliosis, and *A. phagocytophilum* (formerly *Ehrlichia equi*, *E. phagocytophilum*, or the agent of human granulocytic ehrlichiosis) is directly related to the distribution of their shared primary vectors, *Ixodes scapularis* and *Ixodes pacificus* in eastern and western North America, respectively (1). Recent reports have shown that *I. scapularis*, the blacklegged tick, is in numerous locations throughout Canada and appears to be endemic in several regions, including Rondeau Provincial Park, Long Point, and Point Pelee National Park in southwestern Ontario (12–17). Thus, the potentially expanding distribution of *I. scapularis* in Canada may increase the likelihood that dogs and humans will be infected with pathogens primarily transmitted by this vector. Other tick species that can be found throughout southern Ontario and Quebec include *Rhipicephalus sanguineus* (18,19), *Dermacentor variabilis* (12,19–22), *Haemaphysalis leporispalustris* (19,22), *Dermacentor albipictus* (19,20,23), and *Ixodes cookei* (24); however, based upon current knowledge, only the first 2 species are of immediate concern in small animal companion animal medicine. *Rhipicephalus sanguineus*, the primary vector of *E. canis* (25), *Ba. canis* and, possibly, *Bartonella vinsonii* (*berkhoffii*) (3), and possibly *Anaplasma platys* (formerly *Ehrlichia platys*) (26), are closely associated with dog populations throughout the world. All 3 stages of the *R. sanguineus* life cycle (larvae, nymph, adult) feed preferentially on dogs, which results in sustainable tick populations in homes or kennels wherever dogs are present (27). *Dermacentor variabilis*, the primary vector of *Rickettsia rickettsii*, the etiologic agent of Rocky Mountain Spotted Fever (RMSF), is found east of 105° longitude and south of 52° latitude in Canada, which includes portions of Saskatchewan, Manitoba, Ontario, Quebec, New Brunswick, Prince Edward Island, and Nova Scotia, where large, expanding populations have been described (20–22).

Based on the geographic distribution of various tick species, transmission of vector-borne diseases in Canada could potentially include ehrlichiosis, RMSF, babesiosis, bartonellosis, anaplasmosis, and Lyme borreliosis. Although primarily thought to be limited to the warmer North American climates, the geographic distribution of many arthropod vectors is increasing as a result of frequent, widespread human and pet travel, aerial transport by adventitious birds, and changes in the environment, including global warming, that allow tick populations to overwinter (16,28,29). These factors, as well as the important role of dogs as sentinel animals for detecting exposure to tick-borne organisms, underscores the importance of periodic determination of the canine seroprevalence to tick-borne pathogens in Canada. The purpose of this study was to determine the seroprevalence of selected vector-transmitted organisms in southern Ontario and Quebec, based on samples submitted to the Vector Borne Disease Diagnostic Laboratory (VBDDL) at the North Carolina State University College of Veterinary Medicine.

Materials and methods

All available serum samples from dogs in southern Ontario and Quebec submitted to the VBDDL between August 9, 2000, and

September 19, 2003, were included in the study. Samples were submitted for diagnostic testing from clinics by the attending veterinarian. Clinical data available from each dog were limited, but they included breed and location (city and province) of the veterinary hospital from which the sample was submitted. Travel history and reason for submission were not available for most samples.

The specific tests performed on each sample at the VBDDL were dependent on the tests requested by the submitting veterinarian. A standard serological panel consisting of antibodies to *Bo. burgdorferi*, *Ba. canis*, *Bar. vinsonii* (*berkhoffii*), *E. canis*, and *R. rickettsii* might have been requested, as well as individual serologic tests. In addition to the tests requested by the attending veterinarian, antibodies to *A. phagocytophilum* and *Bar. hensalae* were tested for retrospectively by using stored serum for all samples that were submitted for the standard serologic test panel.

Serologic assays

Antibodies against *E. canis*, *R. rickettsii*, *Ba. canis*, *Ba. gibsonii*, *Bar. vinsonii*, *Bar. hensalae*, and *A. phagocytophilum* were determined by the indirect fluorescent antibody (IFA) test, as previously described (1,7,8). Briefly, serum serially diluted to 1:32 was applied to multiple-well microscope slides that contained the affixed antigen of interest. After incubation, washing, and drying, fluorescein isothiocyanate (FITC)-labeled goat anti-dog immunoglobulin (Kirkegaard and Perry Laboratories, Gaithersburg, Maryland, USA) was applied to each well. For positive samples, serial dilutions were used to determine the antibody titer; reciprocal titers ≥ 64 were considered seropositive. *Borrelia burgdorferi* was tested serologically by using a commercially available peptide C6 enzyme-linked immunosorbent assay-based test kit (SNAP® 3Dx™; IDEXX Laboratories, Westbrook, Maine, USA), according to the manufacturer's instructions.

Polymerase chain reaction assay.

Ehrlichia-genus primers were used first to detect DNA from *Ehrlichia* spp. and *Anaplasma* spp., as previously described (30). Positive samples were then analyzed with primers specific for *E. canis*, *E. chaffeensis*, *E. ewingii*, *A. platys*, and *A. phagocytophilum* (30).

Results

A total of 288 samples were submitted to the VBDDL from veterinarians in southern Ontario and Quebec throughout the period of study. Antibodies to *Ba. gibsonii*, *Ba. canis*, *Bar. hensalae*, *R. rickettsii*, and *Bo. burgdorferi* were found most frequently, but there was little serologic evidence to support the presence of *A. phagocytophilum* (0/53), *Bar. vinsonii* (*berkhoffii*) (0/59), and *E. canis* (1/271 [0.37%]), as shown in Table 1. The polymerase chain reaction (PCR) prevalence of *E. canis* was 7.3% (3/38), while 2 of the PCR-positive samples were seronegative for *E. canis* antigens by IFA testing (Table 2). Of the 288 samples, 139 (48%) were submitted for only *E. canis* serologic testing, 54 (18.8%) for the standard tick-borne disease serologic panel, 35 (12%) for *Bo. burgdorferi* and *E. canis* serologic testing,

Table 1. Serological and polymerase chain reaction (PCR) results for selected tick-borne organisms in samples from dogs in southern Ontario and Quebec

Organism	Number of samples (<i>n</i>)	Titer negative	Reciprocal antibody titer			Prevalence
			64–256	256–2048	> 2048	
<i>Babesia canis</i>	66	61	4	1		7.58%
<i>Babesia gibsonii</i>	8	7	1			12.50%
<i>Ehrlichia canis</i>	271	270	1			0.37%
<i>Bartonella hensalae</i>	55	52	3			5.45%
<i>Bartonella vinsonii berkhoffii</i>	59	59				0.00%
<i>Rickettsia rickettsii</i>	68	65	2		1	4.41%
<i>Anaplasma phagocytophilum</i>	53	53				0.00%
<i>Borrelia burgdorferi</i>	108	106	Peptide C6 ELISA-based kit positive = 2			1.85%
<i>Ehrlichia canis</i> PCR	41	38	Positive = 3			7.32%

Table 2. Geographical, historical, serological, and polymerase chain reaction (PCR) results for dogs PCR positive or seropositive for select tick-borne pathogens in southern Ontario

Dog	Breed ^a	City of origin	Pathogen(s)	Serology	PCR	Travel	Clinical history
1	Mix	Guelph, ON	<i>Ehrlichia canis</i>	1:128	—	Africa	Mange
2	Greyhound (18)	Guelph, ON	<i>Babesia canis</i>	1:128	—	New Hampshire, USA	Unknown
3	Greyhound (18)	Guelph, ON	<i>Babesia canis</i>	1:256	—	USA	Unknown
4	Greyhound (18)	Guelph, ON	<i>Babesia canis</i>	1:64	—	USA	Unknown
5	Greyhound (18)	Guelph, ON	<i>Babesia canis</i>	1:64	—	USA	Unknown
6	Greyhound (18)	Guelph, ON	<i>Babesia canis</i>	1:64	—	New Hampshire, USA	Unknown
7	Mix	Blenheim, ON	<i>Babesia gibsonii</i> , <i>B. burgdorferi</i>	1:64, C6 peptide Positive	—	None	Fever, Hemolytic anemia
8	Mix	Blenheim, ON	<i>Bartonella hensalae</i>	≥ 1:64	—	Unknown	Hemolytic anemia
9	American Eskimo (3)	Guelph, ON	<i>Bartonella hensalae</i>	1:128	<i>Ehrlichia</i> PCR negative	None	SLE-like syndrome
10	Golden retriever (16)	Guelph, ON	<i>Bartonella hensalae</i>	1:128	<i>Ehrlichia</i> PCR negative	None	Blastomycosis
11	Bernese mountain dog (4)	Ottawa, ON	<i>Rickettsia rickettsii</i>	1:128	—	None	Chronic renal failure
12	Cock-a-poo (5)	Guelph, ON	<i>Rickettsia rickettsii</i>	1:128	—	Virginia, USA	No clinical signs
13	Belgian Malinois (1)	Guelph, ON	<i>Rickettsia rickettsii</i>	1:4096	—	USA	Protein-losing nephropathy, aortic thromboembolism
14	Nova Scotia duck tolling retriever (5)	Guelph, ON	<i>B. burgdorferi</i> , <i>Ehrlichia canis</i>	C6 peptide positive, Negative	<i>Ehrlichia canis</i> positive	None	Immune-mediated thrombocytopenia
15	Beagle (2)	St. Hyacinthe, QC	<i>Ehrlichia canis</i>	—	<i>Ehrlichia canis</i> positive	Unknown	Unknown
16	Labrador retriever (26)	Guelph, ON	<i>Ehrlichia canis</i>	Negative	<i>Ehrlichia canis</i> positive	None	Immune-mediated thrombocytopenia

^a Number in parenthesis is the total number of samples submitted for the identified breed

14 (4.9%) for *Ehrlichia* genus PCR, 4 (1.4%) for *E. canis*, *Ba. canis*, *Ba. gibsonii*, *Bo. burgdorferi*, and *R. rickettsii* serologic testing, 4 (1.4%) for *E. canis*, *Bo. burgdorferi*, and *R. rickettsii* serologic testing, 3 (1%) for *E. canis* and *Ba. canis* serologic testing, 3 (1%) for *E. canis* and *Bar. vinsonii (berkhoffii)* serologic testing, 3 (1%) for *E. canis*, *Ba. canis*, and *Bo. burgdorferi* serologic testing, 3 (1%) for *E. canis* and *Bar. vinsonii (berkhoffii)* serologic testing, 3 (1%) for *E. canis* and *R. rickettsii* serologic testing, and 23 (8%) for individual serological or PCR tests.

The population of dogs consisted of 72 different breeds and included the Labrador retriever (9%, 26/288), greyhound (6.3%,

18/288), golden retriever (6%, 16/288), cocker spaniel (4.9%, 14/288), and mixed breed (17%, 49/288). Age and gender were not available for the majority of dogs. The breeds with positive serologic or PCR results are described in Table 2.

Many of the samples submitted for analysis were from the province of Ontario with the majority (*n* = 240) from the city of Guelph, of which 235 (82%) were submitted from the Animal Health Laboratory at the Ontario Veterinary College. The remaining submissions were from the cities of Ottawa (*n* = 27), Toronto (*n* = 12), and Blenheim (*n* = 4). Five samples were from the cities of Montreal (*n* = 4) and St. Hyacinthe



Figure 1. Map of Ontario and Quebec indicating cities from which positive and negative serologic and PCR samples originated.

($n = 1$) in Quebec. Overall, 16 different veterinary hospitals submitted samples for analysis during the study period. A map showing the origin of submitted samples is shown in Figure 1.

Samples with positive serologic or PCR test results were submitted mainly from the Animal Health Laboratory at the Ontario Veterinary College in Guelph. Other cities with positive samples included: Blenheim ($n = 2$), 1 seroreactive to *Bar. henselae* ($\geq 1:64$) and the other seroreactive to *Bo. burgdorferi* and *Ba. gibsonii* (1:64) antigens; Ottawa ($n = 1$), antibodies to *R. rickettsii* (1:128); and St Hyacinthe ($n = 1$) PCR positive for *E. canis* (Figure 1).

Travel history was not available for the majority of cases and direct questioning of owners was not possible. Follow-up questioning did determine that the 2 *E. canis* PCR-positive dogs with negative IFA serologic results were native to Ontario and had not traveled outside the province prior to evaluation; the travel history of the additional dog with positive *E. canis* PCR results was unknown. Travel to Africa was documented for the single case seropositive for *E. canis*. Both *Bo. burgdorferi* seropositive dogs (1 also PCR+ for *Ehrlichia*) had not traveled outside their respective provinces. One dog that was *R. rickettsii* seroreactive had traveled to Virginia, a state endemic for RMSF, 1–2 wk prior to sample submission. Two *Ixodes* spp. ticks were found

on the dog at the time of examination and sample submission. Acute and convalescent antibody titers (1:128 and 1:128, respectively) to *R. rickettsii* failed to document seroconversion consistent with a diagnosis of RMSF. Travel to the United States was documented for an additional case seropositive for RMSF (1:4096). Two of the 3 *Bar. henselae* seropositive dogs did not travel outside Canada; travel history for the remaining *Bar. henselae*-positive dog was unknown. All greyhounds seropositive for *Ba. canis* were obtained from the United States. Travel and clinical history are further summarized in Table 2.

Discussion

The overall seroprevalence to a panel of tick-transmitted organisms in dogs from southern Ontario and Quebec is low, based on the findings of the current study. Previous studies of vector-borne disease in dogs from Canada include isolated case reports (31,32) and a study of rural dogs that documented a *R. rickettsii* seroprevalence of 2.5% in Alberta and Saskatchewan (33). Because there is little serologic evidence of tick-transmitted disease in southern Ontario and Quebec, veterinarians should actively pursue the travel history of dogs with suspected tick-borne illness to determine if the dog has visited geographic areas that are endemic for various tick-borne pathogens.

Infection with *Ehrlichia* spp. may cause a variety of clinical signs, ranging from fever, polyarthritis, and thrombocytopenia to asymptomatic infections. Previous seroprevalence rates for *E. canis* of 2.4%, 2.9%, and 6.4% have been found in recent studies conducted in Rhode Island, North Carolina, Virginia, Maryland, and Pennsylvania (1,4,9). The low prevalence of 0.37% in the current study may be explained by several factors, including the absence of *E. canis* in ticks from southern Ontario and Quebec, infrequent exposure of pet dogs to *R. sanguineus*, or inefficient transmission of *E. canis* by *R. sanguineus* in colder climates. While there is evidence that *E. canis*, *E. chaffeensis*, and, to a lesser extent, *E. ewingii* and *A. phagocytophilum* cross-react serologically (4,26,30), there is also evidence that antibodies to 1 strain of *E. canis* may not react similarly to other strains of *E. canis* (30). Thus, if the current study utilized *E. canis* antigens that differed from the endemic strain in a given geographic area, the seroprevalence might be falsely low. Of additional interest is a recent report that describes the presence of *E. canis*-like infection in 3 cats, 2 of which were from southern Ontario (34). Antibodies to available *E. canis* antigens were not detected by IFA testing, but identical ehrlichial DNA was amplified from all 3 cats (100% homologous to 16S rDNA sequences in GenBank). In the current study, the dramatic difference between serologic and PCR prevalence may be related to the presence of a different strain or an *Ehrlichia* sp. that is present in Canada but does not cross-react with currently available *E. canis* antigens. It remains possible, however, that acute infections were documented prior to the development of antibodies to *E. canis*. Based on the discrepancy between *E. canis* serologic and PCR results, the prevalence of ehrlichial or ehrlichial-like infection in dogs in southern Ontario and Quebec may be underestimated, using serologic tests alone. Further research is necessary to determine the significance of the current findings.

The *R. rickettsii* seroprevalence in RMSF endemic areas in the United States has been reported in several studies and ranges from 12.5% to 69% of the dog sera tested (1,7,9). However, antibodies to pathogenic spotted fever group *Rickettsia* spp. cross-react with nonpathogenic *Rickettsia* spp. in a given geographic region being overestimated (7,35). In a previous serosurvey, for instance, the prevalence of RMSF was markedly reduced to an overall prevalence of 5% from 17% to 69% in various geographic locations in North Carolina, after adjusting for cross-reactive antibodies (7). It is likely that the *R. rickettsii* seroprevalence of 4.4% in the current study overestimates the true prevalence of RMSF in southern Ontario and Quebec. Although 1 dog with travel history to Virginia was *R. rickettsii* seroreactive, it did not develop a 4-fold rise in titer in the convalescent sample, which would have been expected after a primary infection; thus, it remains possible that exposure to a *Rickettsia* sp. occurred in Canada, predating travel to Virginia. Because the known vector (*D. variabilis*) is present east of 105° longitude in Canada, the potential for *R. rickettsii* infection exists, and human cases, although infrequent, have been reported in this region (36). Additionally, isolates of *R. rickettsii* have been found in *D. variabilis* from Ontario and Nova Scotia, further

emphasizing the potential for human or canine RMSF in this region (36).

Antibodies to *Ba. canis* were detected only in greyhounds, a breed in which the disease is endemic in the southeastern United States (37,38). Previous studies documented overall seroprevalences of 3.8% for *Ba. canis* and 5.6% for *Babesia* spp. (39,40). Because many *Babesia* spp. infections are subclinical, transportation of dogs is common, and transmission occurs transovarially in the primary vector (*R. sanguineus*), establishment of babesiosis in southern Ontario and Quebec is possible, although the current study does not provide serological evidence of *Ba. canis* infection outside the greyhound population (18,37).

The *Bo. burgdorferi* (1.85%) and *A. phagocytophilum* (0%) seroprevalences in the current study are low compared with those in areas of the northeastern United States, where *I. scapularis*, the shared primary vector, is endemic. For instance, in a report from Rhode Island, where testing was performed by the VBDDL, the seroprevalence of *Bo. burgdorferi* and *A. phagocytophilum* in dogs was 52% and 14.4%, respectively (1). Importantly, the C6 peptide serologic assay (SNAP® 3Dx™, IDEXX Laboratories) for *Bo. burgdorferi* used in the current study is not affected by vaccination (41). Thus, the seropositive samples represent natural exposure to *Bo. burgdorferi*. The lack of travel history of *Bo. burgdorferi*-seropositive dogs and low seroprevalence suggest that disease transmission may be inefficient in this region, possibly secondary to climate or other environmental factors, despite the presence of the vector and organism. However, there is evidence that *Bo. burgdorferi* can be transmitted by *I. scapularis*, the primary vector in southern Ontario and Quebec (13–16). Of the *I. scapularis* ticks collected from dogs across southern Ontario with no history of travel, *Bo. burgdorferi* DNA was amplified from 7/121 (5.8%) ticks, and 9/9 dogs tested were seroreactive to *Bo. burgdorferi* antigens by IFA and western blotting (12). In addition, *A. phagocytophilum* DNA was amplified from *I. scapularis* ticks collected from an endemic region of southern Ontario, providing further evidence that both organisms are present in southern Ontario and Quebec and remain closely associated with *I. scapularis* (42).

Bartonellosis in dogs may be caused by several different *Bartonella* spp. with *Bar. vinsonii* (*berkhoffii*) presumably having a primary pathogenic role. Previous studies have found seroprevalence rates for *Bar. vinsonii* (*berkhoffii*) of 3.6% and 4.7% for sick dogs in North Carolina and Virginia (3,43). Additionally, in a study utilizing military working dogs with frequent tick exposure from across the United States, an overall prevalence of 8.7% was found (44). Importantly, in the military working dog study, the seroprevalence was significantly different among geographic regions, with the southern and northeastern states having higher seropositive rates than the Midwest and the mountain states, which had no seroreactive samples. Possible reasons for the differences include the presence of multiple vectors or a single vector affected by environmental or geographic differences (44). The low seroprevalence of *Bar. vinsonii* (*berkhoffii*) (0%) in the current study may be related to similar factors, including the effects of the environment or the absence of the vector in southern Ontario and Quebec, which, as yet, is not clearly established.

The seroprevalence (5.45%) for *Bar. henselae* in the current study is similar to previously published rates in Hawaii (6.5%) (45), Japan (7.7%) (46), and the United Kingdom (3%) (47), but lower than the prevalence in sick dogs from North Carolina (27%) (43). The presence of antibodies reactive to *Bar. henselae* antigens was associated with *R. rickettsii* and *Bar. vinsonii* (*berkhoffii*) seroreactivity in a previous study (43); however, the current study does not provide evidence for this association, possibly because of the low overall *Bartonella* spp. seroprevalence. Although the current study provides evidence that dogs in southern Ontario and Quebec can have antibodies to *Bar. henselae*, the clinical significance of this finding is unknown.

There are several recent studies documenting the distribution of tick vectors in Canada (12–16,18,21,24). Because the geographic range and distribution of arthropod vectors is likely to change over time through changes in the environment, migrating birds, and travel, the distribution of diseases caused by pathogens transmitted primarily by tick vectors may also change. For example, there is evidence that migratory birds are important in dispersing tick vectors and their associated diseases throughout various geographic areas, including Canada (16,28,29). *Amblyoma americanum* ticks, a species predominantly found in the southeastern United States and the principle vector of human and canine monocytic ehrlichiosis (*E. chaffeensis*), have been removed from birds throughout various locations in Canada (16). Additionally, this tick species has also been recovered from dogs and cats in Ontario with no history of travel (16). Thus, diseases caused by pathogens transmitted primarily by *A. americanum*, while likely uncommon, may occur in new geographic regions, because transient or sustainable populations with their associated pathogens may develop far outside the natural host range (48).

Several important limitations should be considered when examining the results of the current study. Because serum samples were submitted from a proportionally small number of veterinary hospitals from a limited geographic range, the sample population is not representative of the general dog population of southern Ontario and Quebec. The lack of available travel history for some of the test-positive samples may also confound the results, the possibility that tick-exposure occurred in areas other than southern Ontario and Quebec cannot be ruled out. Additionally, samples from animals with a travel history may have been preferentially submitted for evaluation of vector-borne disease by attending veterinarians. The findings of the current study, however, may be used to provide an approximation of the prevalence of selected vector-borne pathogens in southern Ontario and Quebec.

Veterinarians should actively pursue the travel history of dogs with infections suspected of having been caused by tick-borne pathogens while attempting to determine the potential for exposure to tick-borne organisms that are endemic in specific geographic regions. Many tick-borne organisms can induce chronic infections in dogs for months to years before disease manifestations develop. Researchers should also consider the potential that novel tick-borne pathogens may be transmitted by tick species in colder climates that will not be detectable by using currently available serological tests. Widespread serological

and molecular-based studies are needed to definitively determine the prevalence of tick-borne organisms throughout Canada. Furthermore, periodic studies that investigate prevalence and distribution of arthropod vectors in Canada are necessary to assess the dynamics associated with the risk disease caused by tick-borne organisms in dogs and humans. CVJ

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