Brief Communication Communication brève

Seroprevalence of *Borrelia burgdorferi, Anaplasma phagocytophilum, Ehrlichia canis,* and *Dirofilaria immitis* among dogs in Canada

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Abstract — The seropositivity of dogs to *Borrelia burgdorferi, Anaplasma phagocytophilum,* and *Ehrlichia canis* antibodies, and *Dirofilaria immitis* antigen was assessed in Canada. *Borrelia burgdorferi* had the highest seroprevalence, while that of *Dirofilaria immitis* has not changed significantly in the past 20 y. The risk for these vector-borne infectious agents in Canadian dogs is low but widespread with foci of higher prevalence.

Résumé — Séroprevalence de *Borrelia burgdorferi,* d'*Anaplasma phagocytophilum,* d'*Ehrlichia canis* et de *Dirofilaria immitis* chez les chiens au Canada. La séropositivité des chiens aux anticorps de *Borrelia burgdorferi,* d'*Anaplasma phagocytophilum* et d'*Ehrlichia canis* et à l'antigène de *Dirofilaria immitis* a été évaluée au Canada. *Borrelia burgdorferi* présentait la séroprévalence la plus élevée, tandis que celle de *Dirofilaria immitis* n'a pas significativement changé au cours des 20 dernières années. Le risque de transmission de ces agents infectieux par un vecteur chez les chiens canadiens est faible mais répandu avec des foyers de prévalence supérieure.

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The risk of a dog acquiring a canine heartworm or tick-borne infection depends on the prevalence of the pathogen and the abundance of the vector (1). In Canada, the environmental suitability for *Borrelia burgdorferi* and *Anaplasma phagocytophilum* vectors, *Ixodes scapularis* and *Ixodes pacificus,* appears to be growing (2). The 4 major factors which affect the range of *I. scapularis* are habitat suitability, host abundance, dispersal by hosts, and climate (3). Climate change has been postulated to be a major cause of the range expansion of *I. scapularis* northward. According to the 1991 Canadian Consensus on Lyme disease, *I. scapularis* is considered endemic when all 3 feeding stages are present on resident animals for at least 2 consecutive years. In 1991, there was one known location in Canada on the north shore of Lake Erie considered to be endemic for *I. scapularis.* Since then, the number of established populations has increased dramatically to 11 with populations along the north shore of Lake Erie, Thousands Islands National Park along the north shore of Lake Ontario, the southern coast of Nova Scotia, and southeastern Manitoba (3,4). In Quebec, the number of *I. scapularis* reported in their surveillance program has been increasing steadily. Since 2007, *I. scapularis* has been the most common tick in Quebec and it is endemic in multiple locations in the southwest region of the province (5). In western Canada, endemic foci of *I. pacificus* are widespread in southern British Columbia, and surveillance programs indicate that bird-borne *I. pacificus* may also occur outside regions where the tick is established (3,4). The determination of endemic areas is important as they represent an increased risk of contact with disease carried by the ticks. In some very highly endemic regions of the US, more than 50% of the unvaccinated dogs may test positive for *B. burgdorferi* (6).

In many areas of Canada where there are no established populations of *I. scapularis,* it is believed that adventitious ticks are carried into Canada on migratory birds. Approximately 3×10^9 migratory birds enter Canada from the US each year and it has been postulated that they carry between 50 to 175×10^6 *I. scapularis* ticks into Canada every year (3,7). Many of these birds carry ticks that are positive for *B. burgdorferi* and/or *A. phagocytophilum.* An estimated 8% to 12% of the *I. scapularis* ticks carried by migratory birds are positive for *B. burgdorferi,* which represents a geographically widespread, albeit low risk, of acquiring Lyme borreliosis in Canada (8). Apart from a study by Gary et al in 2006 (9), which analyzed samples from 288 canine patients from southern Ontario and Quebec, there are no other published seroprevalence studies of tick-borne diseases in the dog population of Canada.

In contrast, since 1977 there have been multiple studies assessing canine heartworm prevalence in Canada (10). These studies indicate that heartworm prevalence in Canada has been

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Table 1. Geographic distribution of seroprevalence to *Borrelia burgdorferi*, *Anaplasma phagocytophilum*, *Ehrlichia canis*, and *Dirofilaria immitis* among dogs in Canada

Location	Number of practices	Number of samples	Number *D. immitis* positive (%)	Number *E. canis* positive (%)	Number *B. burgdorferi* positive (%)	Number *A. phagocytophilum* positive (%)
British Columbia	5	418	1 (0.24)	3 (0.72)	0 (0)	0 (0)
Alberta	6	584	2 (0.34)	3 (0.51)	1 (0.17)	0 (0)
Saskatchewan	2	582	0 (0)	0 (0)	2 (0.34)	2 (0.34)
Manitoba	32	13 456	58 (0.43)	0 (0)	256 (1.90)	101 (0.75)
Ontario	137	56 943	109 (0.19)	23 (0.04)	270 (0.47)	49 (0.09)
Canada Capital Region	13	5109	7 (0.14)	0 (0)	37 (0.72)	8 (0.16)
Thousand Islands	11	1349	3 (0.22)	0 (0)	38 (2.82)	1 (0.07)
Northern Ontario	6	1971	3 (0.15)	3 (0.15)	18 (0.91)	6 (0.30)
St. Lawrence Seaway	5	1780	5 (0.28)	0 (0)	15 (0.84)	0 (0)
North of Lake Erie	15	9298	31 (0.33)	5 (0.05)	84 (0.90)	9 (0.10)
Greater Toronto area	4	1099	0 (0)	0 (0)	3 (0.27)	2 (0.18)
Quebec	45	13 390	15 (0.11)	9 (0.07)	76 (0.57)	12 (0.09)
Estrie	1	357	0 (0)	0 (0)	2 (0.56)	0 (0)
Laurentians	10	2722	3 (0.11)	1 (0.04)	22 (0.81)	3 (0.11)
Montérégie	8	1907	5 (0.26)	0 (0)	13 (0.68)	4 (0.21)
Greater Montreal area	8	4591	2 (0.04)	5 (0.11)	19 (0.41)	4 (0.09)
New Brunswick	2	151	0 (0)	0 (0)	1 (0.66)	0 (0)
Nova Scotia	8	697	1 (0.14)	1 (0.14)	15 (2.15)	0 (0)
Prince Edward Island	1	30	1 (3.3)	1 (3.3)	3 (10)	0 (0)
Newfoundland	0	0	0 (0)	0 (0)	0 (0)	0 (0)
CANADA	238	86 251	187 (0.22)	40 (0.05)	624 (0.72)	164 (0.19)

relatively stable over the past 20 y at roughly 0.16% (11). The areas with the highest heartworm prevalence are the southern areas of Ontario, Manitoba, and Quebec, and the southern Okanagan Valley (10).

In Canada, documented case reports of Lyme borreliosis, anaplasmosis, and heartworm in domestic animals can be found in published literature (9,10,12). To assess the extent to which canine patients are being infected with vector-borne pathogens in Canada, the seroprevalence of heartworm *(Dirofilaria immitis)* and 3 agents of tick-borne diseases *(Borrelia burgdorferi, Anaplasma phagocytophilum, Ehrlichia canis)* of dogs in Canada was assessed in this study.

In 2008, veterinary practices in Canada were invited to participate in a study of vector-borne pathogens in their practice population of dogs. As part of their spring testing, study participants were required to utilize an enzyme-linked immunosorbent assay (ELISA) (SNAP 4Dx; IDEXX Laboratories, Westbrook, Maine, USA) on canine patient blood samples either in-house or at 1 of 5 reference laboratories (IDEXX Canada Reference Labs, Markham, Ontario; Montreal, Quebec; Calgary, Alberta; Edmonton, Alberta; Langley, British Columbia). In exchange for their contributions, a rebate was provided to participants who submitted a minimum of 30 tests. The study period was from January 1 to August 31, 2008.

Enrolled clinics were supplied with study materials that included study design, instructions, and a data collection sheet. Clinics were instructed to record the results of each patient test (positive and negative) on tracking sheets. For patients with positive test results to any of the 4 analytes, additional information was requested which included clinical information of the patient, known tick contact, travel outside the province, concurrent illness, and age. Upon completion of the data sheets, clinics submitted the results via facsimile to IDEXX Laboratories. No confirmation tests were done on positive cases.

A total of 238 practices reported results from 86 251 dogs over the 8-month study period. Figure 1 provides a summary of the seroprevalence results. Of the 86 251 samples analyzed, 624 (0.72%) tested positive for *B. burgdorferi* antibodies, 187 (0.22%) for heartworm antigen, 164 (0.19%) for *A. phagocytophilum* antibodies, and 40 (0.05%) for *E. canis* antibodies. *Borrelia burgdorferi* was the most geographically widespread and most commonly found infectious agent throughout Canada. The areas of highest prevalence were Manitoba, the Thousand Islands, Nova Scotia, and Prince Edward Island (PEI). Apart from PEI, all these regions have known endemic populations of *I. scapularis. Anaplasma phagocytophilum* positives were found in only 4 provinces; Saskatchewan, Manitoba, Ontario, and Quebec. Manitoba was the province with highest prevalence of *A. phagocytophilum* (0.75%) which was over double the prevalence in the province (Saskatchewan, 0.34%) with the second highest prevalence. The geographical distribution of *B. burgdorferi* and *A. phagocyphilum* positives in Canada appears to follow a similar pattern as in the US, predominantly in the Central and Eastern regions of the continent (13). *Dirofilaria immitis* was geographically widespread throughout Canada, and the 0.22% seroprevalence of heartworm found in this study is consistent with previous estimates of heartworm prevalence in Canada (10). *Ehrlichia canis* was the infectious agent with the lowest prevalence in Canada but it was the most commonly found pathogen in British Columbia and Alberta. No practices in Newfoundland and Labrador participated in the study so no information was collected from dogs in this province.

In addition to the seroassessment, clinical and signalment data were provided for 913 of the patients testing positive for 1 or more vector-borne disease. Figure 2 provides a summary of the patient information. Mean age of positive dogs was 5 y with a range of 8 mo to 17 y. No gender predilection was seen with any of the infectious agents. The majority, 79%, of patients

Table 2. Characteristics of patients seropositive to vector-borne disease

	Number (%)						
	All agents	*Borrelia* only	*Anaplasma* only	*Dirofilaria* only	*Ehrlichia* only	*Borrelia and Anaplasma*	*Borrelia and Dirofilaria*
Positives	913 (100)	558 (61.1)	123 (13.56)	147 (16.1)	34 (3.7)	37 (4.1)	10 (1.1)
Travel history							
Yes	94 (10.3)	45 (8.1)	9 (7.3)	21 (14.3)	10 (29.4)	7 (18.9)	0 (0)
No	724 (79.3)	454 (81.2)	99 (80.5)	114 (77.6)	23 (67.6)	26 (70.3)	6 (60.0)
Unknown	95 (10.4)	59 (10.6)	15 (12.2)	12 (8.2)	1 (2.9)	4 (10.8)	4 (40.0)
Tick contact							
Yes	395 (43.3)	262 (47.0)	51 (41.5)	NA[a]	8 (23.5)	26 (70.3)	3 (30.0)
No	435 (47.6)	249 (44.65)	61 (49.6)	NA	22 (64.7)	10 (27.0)	4 (40.0)
Unknown	83 (9.1)	47 (8.4)	11 (8.9)	NA	4 (11.8)	1 (2.7)	3 (30.0)
Health status							
Sick	125 (13.7)	80 (14.3)	11 (8.9)	18 (12.2)	4 (11.8)	7 (18.9)	2 (20.0)
Not sick	752 (82.4)	460 (82.4)	105 (85.4)	124 (84.4)	28 (82.4)	28 (75.7)	6 (60.0)
Unknown	29 (3.2)	15 (2.7)	3 (2.4)	5 (3.4)	2 (5.9)	2 (5.4)	2 (20.0)
Deceased	7 (0.77)	3 (0.54)	4 (3.3)	0 (0)	0 (0)	0 (0)	0 (0)

[a] NA — not applicable.

testing positive, did not have a travel history outside their native province in the past 6 mo. Dogs testing positive for *E. canis* antibodies were the most likely to have traveled outside of their home province (29.4%). Most dogs that had been exposed to *E. canis* did not have a history of tick contact in the past 6 mo while roughly an equal number of patients testing positive for *B. burgdorferi* and *A. phagocytophilum* had had a history of tick contact.

Overall, 13.7% of the dogs testing positive for 1 of the 4 infectious agents were ill. Dogs co-infected with *B. burgdorferi*/*D. immitis,* and *B. burgdorferi*/*A. phagocytophilum* had the highest likelihood of being ill, 20% and 18.9%, respectively. Of the dogs infected with a single agent, patients testing positive for *B. burgdorferi* seropositivity were the most likely to be ill (14.3%) followed by *D. immitis* (12.2%), *E. canis* (11.8%), and *A. phagocytophilum* (8.9%).

The 77.6% of dogs testing positive for *D. immitis* antigen with no travel history outside of their province of residence is consistent with other studies which have found that the majority of heartworm infected dogs had no travel history outside of Canada (14). The rate of clinically ill dogs that tested positive for heartworm in this study, 12.2%, is much lower than some previous studies where the rate of dogs with clinical signs attributed to heartworm was approximately 23% (14). The reason for the difference is unclear, but could be the result of improved compliance in heartworm wellness screening, biasing the results in our study to a more clinically healthy population and the extensive use of microscopic techniques in the past.

There is a distinct geographic pattern for the 3 tick-borne agents with the highest rates of *B. burgdorferi* in central and eastern Canada. The geographic proximity of these provinces to the highly endemic regions of the northeastern and the upper midwestern US, along with habitat suitability, host abundance, and climate change, are the likely causes of higher prevalence of the diseases. The highest rates of *A. phagocytophilum* were found in Manitoba and Saskatchewan. As with *B. burgdorferi,* habitat suitability, host abundance, and climate change, and their proximity to the upper midwest, which has the highest rates of *A. phagocytophilum* in the US are the most likely causes of the higher prevalence in these centrally located provinces. Rates of *E. canis* were very low in Canada and many were associated with travel to endemic regions of the US or Mexico.

Although the sensitivity and specificity of the diagnostic tests are very high for each of the 4 infectious pathogens, it should be noted that with any test, the probability of false positives increases in areas where the agent is uncommon. Positive predictive value is a simple calculation which may give an indication of the potential for false positives. The predictive value is limited by the assumed prevalence of an agent in a population within a given area and the accuracy of the "Gold Standard" test utilized to determine the specificity of a test. For example, if a newer test is more accurate than the agreed upon Gold Standard, the specificity of the newer test may be falsely lowered. In this study, the prevalence of *B. burgdorferi* is 0.72%, and the minimum specificity is at least 99.6% (15). From these data, we may assume that at least 2/3 of Lyme positives are true positives. The prevalence of the other infectious agents in this study was less than Lyme so there is the possibility that their rates of false positives may be higher. It is generally recommended that clinically normal positive dogs from low prevalence areas be re-tested before initiating treatment (13).

This study shows that the risk for vector-borne infectious agents in the Canadian canine population is low but widespread with foci of higher prevalence. *Borrelia burgdorferi* was the agent with the highest seroprevalence in Canada while the seroprevalence of heartworm in Canada has not changed significantly in the past 20 y even with the widespread use of preventative treatments. Expansion of *I. scapularis* into Canada may result in an increased risk of infection of the canine population to tick-borne infectious agents such as *B. burgdorferi* and *A. phagocytophilum.* CVJ

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