Serological survey of canine vector-borne diseases in Saskatchewan, Canada

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Abstract — Whole blood samples were collected from 515 dogs in the practice region surrounding Saskatoon, Saskatchewan, Canada between 2008 and 2010 and evaluated for seroprevalence of vector-borne diseases. Of 515 samples, 12 (2.3%) were positive, with 7 (1.4%) positive for antibodies to *Borrelia burgdorferi*. These prevalences are higher than those previously reported for this region.

Résumé — Enquête sérologique des maladies canines à transmission vectorielle en Saskatchewan, au Canada. Des échantillons de sang total ont été prélevés auprès de 515 chiens dans des établissements vétérinaires des environs de Saskatoon, en Saskatchewan, au Canada, entre 2008 et 2010, et ont été évalués pour la séroprévalence des maladies à transmission vectorielle. Parmi les 515 échantillons, 12 (2,3 %) étaient positifs et 7 (1,4 %) étaient positifs pour les anticorps contre *Borrelia burgdorferi*. Ces prévalences sont supérieures à celles précédemment signalées pour cette région.

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Vector-borne diseases are an important and emerging health concern for humans and animals worldwide. A recent special report in the *Canadian Veterinary Journal* highlighted the increasing risk of Lyme disease in Canada (1). The geographic distribution of vectors, reservoir hosts, and pathogens has been shifting and expanding (2,3), necessitating up-to-date surveillance studies to assess current risk.

Several large seroprevalence studies have provided excellent data on changing pathogen prevalence in North America and the Caribbean; however, these studies lack strong data from the Canadian prairie provinces of Alberta, Saskatchewan, and Manitoba (4–6). One recent short communication described the seroprevalence of vector-borne diseases in a large number of dogs from across Canada; however, less than 2% of the cases came from the provinces of Saskatchewan and Alberta (7). A more recent large survey that reviewed 115 636 SNAP 4DX Plus test results collected in 2013–2014 from eastern Canada west to Saskatchewan found that 2.5% of tests were positive for *Borrelia burgdorferi* antibodies, while less than 0.5% of samples were positive for antibodies to *Ehrlichia canis* or *Anaplasma phagocytophilum*. Despite this large number of samples, only 186 samples came from Saskatchewan, with 0.5% of those testing positive for antibodies to *B. burgdorferi* (8). While the western Canadian provinces of British Columbia and Manitoba have well-recognized pockets of endemic vector-borne diseases including heartworm and Lyme, Saskatchewan and Alberta continue to be underrepresented in the literature (7–9).

Three dogs with clinical illness due to granulocytic anaplasmosis were identified in Saskatchewan in 2009 and seroprevalence data identifying exposure to West Nile virus in dogs from Saskatchewan were reported in 2015; however, other vector-borne diseases such as ehrlichiosis and Rocky Mountain spotted fever are not usually considered endemic in Saskatchewan (10,11). While there are no known endemic populations of *Ixodes scapularis* within the province of Saskatchewan, the role of migratory birds in the delivery of competent vectors to the region as well as the projected expansion of vector range due to climate change make further investigation critical to allow for the development of appropriate screening and prevention strategies in this region (12–14).

The aim of this study was to determine prevalence of *B. burgdorferi, A. phagocytophilum, E. canis,* and *Dirofilaria immitis;* and to identify potential risk factors for exposure to vector-borne pathogens in dogs from Saskatchewan.

Serum and whole blood samples were collected from 135 clinically healthy client-owned dogs presenting to the Veterinary Medical Centre (VMC) in Saskatoon, Saskatchewan, Canada between 2008 and 2010 for routine health care procedures.

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Disclosure statement

IDEXX Labs Canada provided the SNAP 4Dx tests. There are no competing financial interests.

Table 1. SNAP 4Dx test results. Percent positive test results for dogs tested between 2008–2010 for antigen to *Dirofilaria* immitis and antibodies to *B. burgdorferi, Anaplasma* spp. and *Ehrlichia* spp.

Group	Total positive	*B. burgdorferi*	*Anaplasma* spp.	*Ehrlichia* spp.	*B. burgdorferi* + *Anaplasma* spp.
All samples	12/515 (2.3%)	7/515 (1.4%)	3/515 (0.6%)	3/515 (0.6%)	1/515 (0.2%)
Laboratory samples[a]	7/366 (1.9%)	4/366 (1.1%)	1/366 (0.3%)	3/366 (0.8%)	1/366 (0.3%)
Known total[b]	5/149 (3.4%)	3/149 (2%)	2/149 (1.3%)	—	—
Known healthy[c]	3/135 (2.2%)	1/135 (0.7%)	2/135 (1.5%)	—	—
Known sick[d]	2/14 (14.3%)	2/14 (14.3%)	—	—	—

[a] Samples collected from PDS with no known historical, travel, or clinical information.
[b] Samples with known historical, travel, and clinical information. Healthy and sick dogs combined.
[c] Samples with known historical, travel, and clinical information. Only clinically healthy dogs.
[d] Samples with known historical, travel, and clinical information. Only clinically sick dogs.

Owners signed a consent form approved by the University of Saskatchewan Animal Use Committee to allow testing of their samples. The seroprevalence of antibodies to West Nile virus in these dogs has previously been reported (11). In addition to those 135 healthy dogs, samples were collected during the same time frame from 14 dogs presenting to the VMC for assessment of clinical illness with differential diagnoses including infectious or vector-borne diseases. The final diagnoses for these patients included endocarditis (1/14), lameness/arthritis (2/14), Shar Pei fever (1/14), gastroenteritis (3/14), polyarthritis (5/14), unspecified immune mediated disease (1/14), fever of unknown origin (2/14), arthritis (1/14), with 1 dog having both fever and lameness. None of the 135 healthy client-owned dogs or the 14 clinically ill dogs had left the province of Saskatchewan in the previous 2 y.

Additionally, 366 whole blood samples submitted for laboratory testing to Prairie Diagnostic Services Laboratory, Saskatoon, Saskatchewan were evaluated for seroprevalence of the 4 previously described pathogens. No historical data, travel, or medical information was available for these samples.

The SNAP 4Dx (IDEXX Laboratories, Markham, Ontario) was used to determine the presence of *D. immitis* antigen, *A. phagocytophilum* antibody, *B. burgdorferi* antibody, and *E. canis* antibody in each sample. There may be cross reactivity with *Anaplasma platys* or *Ehrlichia ewingii,* though no further testing was performed to assess for this possibility. The sensitivity and specificity of each of these tests have been reported by IDEXX Laboratories (package insert) and also reviewed previously (8).

Twelve of the 515 samples that were tested (2.3%) had a positive result on the SNAP 4Dx, for 1 or more pathogens, with 1 dog being positive for both *B. burgdorferi* and *A. phagocytophilum* antibodies. Seven of the dogs (1.4%) were positive for *B. burgdorferi* antibodies, 3 (0.6%) tested positive for *A. phagocytophilum* antibodies and 3 (0.6%) were positive for *E. canis* antibodies. No samples were positive for *D. immitis* antigen. The SNAP 4Dx results are summarized in Table 1.

When dogs with a known clinical history were considered separately, 5/149 (3.4%) were seropositive for 1 or more pathogens on the SNAP 4Dx test. These included 3/149 (2.0%) testing positive for *B. burgdorferi* antibodies and 2/149 (1.3%) testing positive for *A. phagocytophilum* antibodies. Dogs classified as sick were more likely to test positive for *B. burgdorferi* antibodies (2/14; 14.3%, both diagnosed with polyarthritis) than were healthy dogs (1/135; 0.7%). None of the sick dogs were positive for *A. phagocytophilum* antibodies. However, 2/135 (1.5%) and 1/135 (0.7%) samples from healthy dogs were positive for *A. phagocytophilum* and *B. burgdorferi* antibodies, respectively.

Seven of the 366 (1.9%) dogs with unknown historical or clinical data collected from the PDS laboratory were positive for 1 or more pathogens on the SNAP 4Dx test. The positive tests included 4/366 (1.1%) positive for *B. burgdorferi* antibodies, 1/366 (0.3%) positive for *A. phagocytophilum* antibodies and 3/366 (0.8%) positive for *E. canis* antibodies. One dog (0.3%) was positive for both *B. burgdorferi* and *A. phagocytophilum* antibodies.

The overall vector-borne disease seroprevalence of 2.3% is higher than has been previously reported for Saskatchewan, with higher seroprevalence rates for *B. burgdorferi* (1.4%) and *A. phagocytophilum* (0.6%) than those reported for the province in recent surveys. The failure to identify any heartworm antigen is consistent with previous reports for the region (7,8). Bowman et al (6) reported the prevalence of vector-borne diseases in the neighboring states of Montana and North Dakota in 2008. Animals from Montana that were tested did not have antibodies to *B. burgdorferi, A. phagocytophilum,* or *E. canis,* but 0.6% had evidence of *D. immitis* antigen, while animals from North Dakota had higher rates for both *B. burgdorferi* (3%) and *A. phagocytophilum* (2.4%) (6).

Interestingly, Herrin et al (8) reported Saskatchewan as having the highest seroprevalence of *E. canis* of any province in Canada with 3/186 (1.6%) samples positive. Our result of 0.6% is lower; however, it is within their reported 95% confidence interval (CI) and is still above the national average reported in that study (0.14%). Herrin et al (8) did not include samples from Alberta or British Colombia in their study, so the true current Canadian national prevalence is unknown.

Although *B. burgdorferi* and *A. phagocytophilum* infections are transmitted by the same tick vector, *Ixodes scapularis,* a much lower seroprevalence was observed for *A. phagocytophilum* antibodies than for *B. burgdorferi* antibodies in this study. This finding is noteworthy given the previous report of clinical anaplasmosis in 3 dogs from Saskatchewan (10). Despite this low value, 0.6% prevalence for *A. phagocytophilum* antibodies is still above what was recently reported to be the national average for Canada (8).

No statistically significant differences were identified between patients with known *versus* unknown clinical histories or

between sick *versus* healthy dogs, despite a tendency for dogs with polyarthritis to be *B. burgdorferi* positive. No risk factors for seropositivity were identified. The association between each evaluated risk factor of interest and serological outcomes was examined using logistic regression (SAS for Windows ver. 9.3; SAS, Cary, North Carolina, USA). The failure to demonstrate a clear difference is likely a reflection of the low positive rate and the small number of patients in the known clinical history and sick patient groups.

There are several important limitations to this study. Only 149/515 dogs had known clinical and travel histories. As such, it is impossible to know the reason for sampling in the 366 dogs tested through the commercial laboratory. These samples may have been submitted as part of routine screening in healthy patients or part of a clinical investigation into underlying disease. Additionally, for the dogs with unknown travel histories, it is possible that they were exposed to pathogens outside of Saskatchewan and not locally exposed. This being said, the seroprevalence rates in this population of dogs are comparable to those found in the 2 recent Canadian studies (7,8) as well as the seroprevalence rates identified in dogs with a known clinical history in the current study, suggesting they may be representative of the population as a whole.

The inclusion of samples from the 14 sick dogs may bias the results towards a higher seroprevalence; however, the authors felt it was appropriate to keep these data in the final analysis in light of the unknown clinical histories of 366 dogs tested through a commercial laboratory. Moreover, when a final analysis was performed after removing data from the 14 sick dogs, the prevalence rate was higher than had been previously reported for Saskatchewan. The small number of sick dogs with clinical signs consistent with vector-borne diseases makes it impossible to draw conclusions about the true prevalence of these diseases in the region. Continued evaluation of the population of sick dogs is necessary to gauge the real impact of vector-borne diseases in the area.

This study used a convenience sample consisting of clinically healthy dogs recruited through the Veterinary Medical Centre. Because it was not a random sample, it is not possible to say how well the results can be generalized to all dogs in the province. It is possible that the independence assumption was violated, because some dogs could have lived in the same household. However, given the average number of dogs per household in the dataset would be substantially less than two, the impact on study estimates of prevalence would be minimal (15).

Given the low prevalence of all of these diseases in Saskatchewan, it must be considered that some of these positive results could represent false positives. The positive predictive value of the SNAP 4Dx test has, however, been suggested to be acceptable in similar serosurveys. Despite this, any asymptomatic dog found to be positive in such a low prevalence environment should be retested prior to clinical intervention (7).

The results from this study serve to expand the information on vector-borne disease in the province of Saskatchewan, and suggest that these diseases must remain in consideration when clinical signs are present, despite lack of travel to a previously documented endemic area.

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References

1. Bouchard CB, Leonard E, Koffi JK, et al. The increasing risk of Lyme disease in Canada. Can Vet J 2015;56:693–699.
2. Chomel B. Tick-borne infections in dogs: An emerging infectious threat. Vet Parasitol 2001;179:294–301.
3. Ogden NH, Mechai S, Margod G. Changing geographic ranges of ticks and tick-borne pathogens: Drivers, mechanisms and consequences for pathogen diversity. Front Cell Infect Microbiol 2013;3:46.
4. Yancey CB, Hegarty BC, Qurollo BA, et al. Regional seroreactivity and vector-borne disease co-exposure in dogs in the United States from 2004–2010: Utility of canine surveillance. Vector-borne Zoonotic Dis 2014;14:724–732.
5. Qurollo BA, Chandrashekar R, Hegarty BC, et al. A serological survey of tick-borne pathogens in dogs in North America and the Caribbean as assessed by *Anaplasma phagocytophilum, A. platys, Ehrlichia canis, E. chaffeensis, E. ewingii* and *Borrelia burgdorferi* species-specific peptides. Infect Ecol Epidemiol 2014;4. doi: 10.3402/iee.v4.24699.
6. Bowman D, Little SE, Lorentzen L, Shields J, Sullivan MP, Carlin EP. Prevalence and geographic distribution of *Dirofilaria immitis, Borrelia burgdorferi, Ehrlichia canis,* and *Anaplasma phagocytophilum* in dogs in the United States: Results of a national clinic-based serologic survey. Vet Parasitol 2009;160:138–148.
7. Villeneuve A, Goring J, Marcotte L, Overvelde S. Seroprevalence of *Borrelia burgdorferi, Anaplasma phagocytophilum, Ehrlichia canis,* and *Dirofilaria immitis* among dogs in Canada. Can Vet J 2011;52:527–530.
8. Herrin BH, Peregrine AS, Goring J, Beall MJ, Little SE. Canine infection with *Borrelia burgdorferi, Dirofilaria immitis, A. phagocytophilum,* and *E. canis* in Canada, 2013–2014. Parasit Vectors 2017;10:244 doi: 10.1186/s13071-017-2184-7.
9. Banerjee S, Stephen C, Fernando K, Coffey S, Dong M. Evaluation of dogs as sero-indicators of the geographic distribution of Lyme borreliosis in British Columbia. Can Vet J 1996;37:168–169.
10. Cockwill KR, Taylor SM, Snead EC, et al. Granulocytic anaplasmosis in three dogs from Saskatoon, Saskatchewan. Can Vet J 2009;50:835–840.
11. Gaunt MC, Waldner C, Taylor SM. Serological survey of West Nile Virus in pet dogs from Saskatchewan, Canada. Vector-borne Zoonotic Dis 2015;15:755–758.
12. Anstead CA, Chilton NB. Ticks feeding on northern pocket gophers *(Thomomys talpoides)* in central Saskatchewan and the unexpected detection of *Ixodes scapularis* larvae. J Vector Ecol 2011;36:355–360.
13. Ogden NH, St-Onge L, Barker IK, et al. Risk maps for range expansion of the Lyme disease vector, *Ixodes scapularis,* in Canada now and with climate change. Int J Health Geogr 2008;7:24. doi: 10.1186/1476-072X-7-24.
14. Ogden NH, Barker IK, Francis CM, Heagy A, Lindsay LR, Hobson KA. How far north are migrant birds transporting the tick *Ixodes scapularis* in Canada? Insights from stable hydrogen isotope analysis of feathers. Ticks Tick Borne Dis 2015;6:715–720.
15. Dohoo I, Martin W, Stryhn H. Introduction to clustered data. In: Veterinary Epidemiologic Research. 2nd ed. Charlottetown, PEI: VER Inc., 2010:535.