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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

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Introduction: Tick-borne pathogens cause a spectrum of disease manifestations in both dogs and humans. Recognizing regional and temporal shifts in exposure are important as tick distributions change. To better delineate regional exposure to canine tick-borne pathogens, an expanded set of species-specific peptides were used to detect *Anaplasma phagocytophilum* (*Aph*), *Anaplasma platys* (*Apl*), *Ehrlichia canis* (*Ec*), *Ehrlichia chaffeensis* (*Ech*), *Ehrlichia ewingii* (*Eew*), and *Borrelia burgdorferi* (*Bb*) antibodies in canine serum.
Methods: Archived canine serum samples ($n = 6{,}582$) collected during 2008–2010 and in 2012 from the US, Canada, and the Caribbean were retrospectively screened for antibodies against *Ehrlichia* and *Anaplasma* species-specific peptides. Overall, regional and temporal seroprevalence rates were determined.
Results: Overall *Bb* and *Eew* were the most seroprevalent pathogens. During 2008–2010, seroprevalence rates increased overall for *Aph* and *Ech,* and regionally, *Bb* and *Aph* seroprevalence rates increased in the South. Canada had unexpectedly high seroprevalence rates for *Ec* and *Apl*. The most common co-exposures were *Eew* + *Ech*, followed by *Aph* + *Bb* and *Eew* + *Bb*.
Conclusions: This study demonstrated significant shifts in canine vector-borne disease seroprevalence rates. The use of specific peptides facilitated improved geographic delineation of tick-borne pathogen distributions among dogs, which may enhance epidemiological surveillance of vector-borne pathogens shared by dogs and humans.

Keywords: *canine vector-borne disease*; *Lyme disease*; Anaplasma; Ehrlichia; Borrelia burgdorferi; *seroprevalence*

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Canine vector-borne diseases (CVBDs) are prevalent in the US, Canada, and the Caribbean. Tick-borne pathogens, including *Anaplasma phagocytophilum* (*Aph*), *Anaplasma platys* (*Apl*), *Ehrlichia canis* (*Ec*), *Ehrlichia chaffeensis* (*Ech*), *Ehrlichia ewingii* (*Eew*), and *Borrelia burgdorferi* (*Bb*), infect dogs and humans, resulting in clinical or subclinical infections (1–7). As tick distributions change through ecosystem fluctuations, wildlife migration, and increased international transport of companion animals, diagnosing and managing dog and human tick-borne diseases has become medically complex and more challenging. Previous studies indicate that *Bb* seroreactive dogs are effective sentinels for human Lyme disease risk (7, 8). Recognizing risk factors and the prevalence of single and co-exposures within a particular region is epidemiologically important for public health and diagnostically important for clinicians. Spatio-temporal tick-borne pathogen surveillance should identify high-risk

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areas for vector-borne pathogen exposure, facilitate the diagnosis of regionally neglected pathogens, and better elucidate co-infection risks.

In 2001, IDEXX Laboratories, Inc., developed rapid, in-house ELISA platforms (SNAP®3Dx®, SNAP®4Dx®, and SNAP® 4Dx® Plus), allowing veterinarians to screen for CVBDs (heartworm disease, Lyme disease, ehrlichiosis, and anaplasmosis). Species-specific peptides developed to detect canine antibodies to *Ec, Ech, Eew, Aph*, and *Apl* were used to manufacture a proprietary, research prototype ELISA SNAP assay (SNAP M-A), showing seroreactivity to individual *Anaplasma* spp. and *Ehrlichia* spp. (9–12). Archived canine serum samples submitted between 2008 and 2010 and in 2012 by veterinarians from dogs with suspected tick-borne disease to the Vector-Borne Disease Diagnostic Laboratory at North Carolina State University (VBDDL–NCSU) were tested using the SNAP M-A. Regional and temporal seroprevalences within the US, Canada, and the Caribbean and common co-exposures between these pathogens are reported.

Methods

Canine serum samples

Archived canine serum samples ($n = 6{,}582$) submitted to the VBDDL–NCSU for serological testing against tick-borne pathogens between January 2008–December 2010 ($n = 6{,}270$; 95.3%) and January–March 2012 ($n = 312$; 4.7%) were available for SNAP M-A testing and analysis. Samples submitted from the same dog within 5 weeks of the initial submission were excluded. Available information included signalment (age, breed, and sex), date of sample collection, and owner or veterinary practice address. Regions, states, and provinces are defined in Table 1.

Serology

All canine sera were retrospectively tested by SNAP M-A for the simultaneous and individual detection of specific *Ec*, *Ech*, *Eew*, *Aph*, *Apl*, and *Bb* antibodies. Included on SNAP M-A are two additional spots containing a combination of *Anaplasma* spp. synthetic peptides, labeled A-genus, and *Ehrlichia* spp. (*Ec* and *Ech* only) synthetic peptides, labeled E-genus. SNAP M-A uses a reversible chromatographic flow of sample and automatic, sequential flow of wash solution and enzyme substrate. Archived canine serum stored at -80°C was thawed to room temperature prior to mixing four drops of serum with 4–5 drops of SNAP M-A conjugate. The mixture was allowed to move across a flow matrix where peptide-specific antibody could bind to peptide-HRP conjugate before color reactant release. Color development indicating a positive reaction was read after 15 min.

Statistical analysis

Seroprevalence, defined as the number of seropositive samples divided by the number of samples tested, was calculated by region, month, and year. The Chi-squared test or Fisher exact test was used to determine significant differences in the proportions of seroreactivity by region, month, and year. Multiple comparisons were performed using the Multtest procedure in SAS/STAT v.9.3 (SAS Institute, Cary, NC). Regions were assigned into the following categories based on owner or veterinary hospital address: Northeast, Mid-Atlantic, South, Midwest, West, Canada, and the Caribbean region, which includes all countries and territories in and around the Caribbean Sea. State-wide seroprevalence was calculated for states with at least 30 sample submissions and depicted in heat maps (openheatmap.com). The proportion of co-exposures, defined as the number of dogs with two or more seropositive results divided by the total number of dogs, was calculated. The following positive species-specific peptide combinations were not considered co-exposures: E-genus + *Ech*, E-genus + *Ec*, A-genus + *Apl*, or A-genus + *Aph*. Odds ratios (ORs) and 95% confidence intervals (95% CI) were used as measures of association between exposure to one pathogen and exposure to a second pathogen (representing concurrent or sequential co-exposures). Level of significance was established at $p < 0.05$. Statistical analyses were performed using SAS/STAT 9.3 (SAS Institute Inc., Cary, NC).

Results

A total of 6,582 dog serum samples were tested, including 6,268 (95.2%) from the US, representing 43 states; 285 (4.3%) from Canada, representing seven provinces; and 29 (0.44%) from the Caribbean region (Table 1). Exposure to at least one tick-borne pathogen was documented in 1,198 (18.2%) dogs. Of the 6,582 sera tested, exposures included *Bb* ($n = 545$, 8.3%), *Eew* ($n = 251$, 3.8%), *Aph* ($n = 227$, 3.4%), *Ech* ($n = 202$, 3.1%), *Ec* ($n = 117$, 1.8%), and *Apl* ($n = 99$, 1.5%) (Table 1). E-genus and A-genus antibodies were detected in 327 (5.0%) and 238 (3.6%) dogs, respectively. Of the E-genus and A-genus antibody positives, 50 (15.3%) and 32 (13.4%) dogs, respectively, did not have species-specific antibodies, which could represent dogs with low *Ehrlichia* and *Anaplasma* species-specific antibody titers or potentially, seroreactivity to a species, such as *Ehrlichia muris* or the Panola mountain *Ehrlichia* not specifically tested for in this study.

Seroprevalences by region are reported in Table 1. The greatest proportion of samples were submitted from the South ($n = 3{,}011$, 45.7%), followed by the Midwest ($n = 1{,}162$, 17.7%), the Mid-Atlantic ($n = 1{,}065$, 16.2%), the Northeast ($n = 532$, 8.1%), the West ($n = 498$, 7.6%), Canada ($n = 285$, 4.3%), and the Caribbean ($n = 29$, 0.44%). Regional comparisons documented significantly higher *Bb* exposure frequencies in the Northeast ($n = 122$, 22.9%)

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Table 1. Distribution of all samples collected between 2008–2010 and 2012 ($n = 6{,}582$) by region (gray shading) and state with seroreactivity to *Ehrlichia canis* (*Ec*), *E. ewingii* (*Eew*), *E. chaffeensis* (*Ech*), *Anaplasma platys* (*Apl*), *A. phagocytophilum* (*Aph*), *Borrelia burgdgorferi* (*Bb*), *Anaplasma* spp. (A-genus), and *Ehrlichia* spp. (E-genus)

Sample origin	Sample #	Eew (%)	Ech (%)	Ec (%)	Apl (%)	Aph (%)	Bb (%)	A-genus (%)	E-genus (%)
Overall	**6,582**	**251 (3.8)**	**202 (3.1)**	**117 (1.8)**	**99 (1.5)**	**227 (3.5)**	**545 (8.3)**	**238 (3.6)**	**327 (5)**
South	3,011	156 (5.2)	129 (4.3)	70 (2.3)	59 (2)	64 (2.1)	100 (3.3)	80 (2.7)	209 (6.9)
FL	501	15 (3)	11 (2.2)	13 (2.6)	12 (2.4)	10 (2)	8 (1.6)	19 (3.8)	21 (4.2)
GA	162	12 (7.4)	6 (3.7)	2 (1.2)	1 (0.6)	3 (1.9)	6 (3.7)	3 (1.9)	6 (3.7)
NC	1,014	104 (10.3)	96 (9.5)	19 (1.9)	22 (2.2)	25 (2.5)	55 (5.4)	26 (2.6)	120 (11.8)
SC	93	8 (8.6)	7 (7.5)	0	1 (1.1)	1 (1.1)	5 (5.4)	2 (2.2)	7 (7.5)
AL	40	1 (2.5)	0	3 (7.5)	1 (2.5)	0	0	0	2 (5)
AR	36	2 (5.6)	1 (2.8)	0	0	0	0	0	2 (6)
KY	69	3 (4.3)	2 (2.9)	0	1 (1.4)	0	4 (5.8)	1 (1.4)	5 (7.2)
LA	27	1 (3.7)	1 (3.7)	1 (3.7)	0	0	0	1 (3.7)	1 (3.7)
MS	16	0	0	0	1 (6.3)	0	0	0	1 (6.3)
OK	42	6 (14.3)	0	1 (2.4)	0	1 (2.4)	0	1 (2.4)	4 (9.5)
TN	45	1 (2.2)	0	1 (2.2)	1 (2.2)	3 (6.7)	2 (4.4)	0	4 (8.9)
TX	966	3 (0.3)	5 (0.5)	30 (3.1)	19 (2)	21 (2.2)	20 (2.1)	27 (2.8)	36 (3.7)
Mid-Atlantic	1,065	61 (5.7)	59 (5.5)	9 (0.8)	12 (1.1)	58 (5.4)	236 (22.2)	55 (5.2)	60 (5.6)
VA	656	45 (6.9)	32 (4.9)	6 (0.9)	7 (1.1)	28 (4.3)	133 (20.3)	30 (4.6)	38 (5.8)
MD	313	12 (3.8)	25 (8)	3 (1)	4 (1.3)	25 (8)	78 (24.9)	21 (6.7)	20 (6.4)
DE	4	0	0	0	0	0	1 (25)	0	0
DC	90	4 (4.4)	2 (2.2)	0	1 (1.1)	5 (5.6)	24 (26.7)	4 (4.4)	2 (2.2)
WV	2	0	0	0	0	0	0	0	0
Northeast	532	18 (3.4)	5 (0.9)	3 (0.6)	8 (1.5)	69 (13)	122 (22.9)	54 (10.2)	10 (1.9)
CT	48	1 (2.1)	2 (4.2)	2 (4.2)	0	12 (25)	16 (33.3)	11 (22.9)	3 (6.3)
MA	35	0	0	0	1 (2.9)	5 (14.3)	14 (40)	4 (11.4)	0
ME	4	0	0	0	1 (25)	0	1 (25)	0	0
NH	19	0	0	0	0	1 (5.3)	5 (26.3)	1 (5.3)	0
VT	6	0	0	0	0	2 (33.3)	2 (33.3)	1 (16.7)	0
NJ	12	0	0	0	0	1 (8.3)	4 (33.3)	1 (8.3)	0
NY	205	10 (4.9)	2 (1)	0	1 (0.5)	33 (16.1)	35 (17.1)	24 (11.7)	4 (2)
PA	203	7 (3.4)	1 (0.5)	1 (0.5)	5 (2.5)	15 (7.4)	45 (22.2)	12 (5.9)	3 (1.5)
Midwest	1,162	14 (1.2)	8 (0.7)	6 (0.5)	7 (0.6)	22 (1.9)	66 (5.7)	31 (2.7)	20 (1.7)
MI	20	0	0	0	0	0	0	0	1 (5)
OH	430	2 (0.5)	1 (0.2)	1 (0.2)	3 (0.7)	3 (0.7)	16 (3.7)	7 (1.6)	5 (1.2)
IN	93	1 (1.1)	1 (1.1)	0	0	2 (2.2)	9 (9.7)	3 (3.2)	2 (2.2)
IL	383	4 (1)	2 (0.5)	1 (0.3)	4 (1)	7 (1.8)	23 (6)	12 (3.1)	5 (1.3)
WI	58	0	0	0	0	6 (10.3)	7 (12.1)	5 (8.6)	0
MN	8	0	0	1 (12.5)	0	2 (25)	2 (25)	1 (12.5)	0
IA	78	3 (3.8)	0	0	0	1 (1.3)	9 (11.5)	2 (2.6)	1 (1.3)
MO	36	2 (5.6)	3 (8.3)	1 (2.8)	0	0	0	0	4 (11.1)
KS	53	2 (3.8)	1 (1.9)	1 (1.9)	0	1 (1.9)	0	0	1 (1.9)
NE	3	0	0	1 (33.3.)	0	0	0	1 (33.3)	1 (33.3)
West	498	2 (0.4)	1 (0.2)	12 (2.4)	5 (1)	10 (2)	15 (3)	6 (1.2)	10 (2.0)
AZ	15	1 (6.7)	0	0	0	0	1 (6.7)	0	0
CA	121	0	0	0	0	4 (3.3)	3 (2.5)	1 (0.8)	0
CO	246	1 (0.4)	0	9 (3.7)	3 (1.2)	5 (2)	10 (4.1)	4 (1.6)	8 (3.3)
NM	61	0	1 (1.6)	1 (1.6)	0	1 (1.6)	1 (1.6)	0	1 (1.6)
NV	5	0	0	0	0	0	0	0	0
OR	35	0	0	0	1 (2.9)	0	0	0	0
UT	2	0	0	1	0	0	0	0	1
WA	12	0	0	0	0	0	0	0	0
WY	1	0	0	1	1	0	0	1	0

Table 1 (*Continued*)

Sample origin	Sample #	Eew (%)	Ech (%)	Ec (%)	Apl (%)	Aph (%)	Bb (%)	A-genus (%)	E-genus (%)
Canada	285	0	0	9 (3.2)	5 (1.8)	3 (1.1)	6 (2.1)	5 (1.8)	9 (3.2)
BC	53	0	0	1 (1.9)	1 (1.9)	0	1 (1.9)	1 (1.9)	1 (1.9)
AB	48	0	0	0	1 (2.1)	0	1 (2.1)	0	1 (2.1)
MB	4	0	0	0	0	0	0	0	0
ON	166	0	0	6 (3.6)	3 (1.8)	3 (1.8)	4 (2.4)	4 (2.4)	6 (3.6)
PE	1	0	0	0	0	0	0	0	0
QC	6	0	0	1 (16.7)	0	0	0	0	1 (16.7)
SK	7	0	0	1 (14.3)	0	0	0	0	0
Caribbean	29	0	0	8 (27.6)	3 (10.3)	1 (3.4)	0	7 (24.1)	9 (31)
*p		<0.0001	<0.0001	<0.0001	0.0249	<0.0001	<0.0001	<0.0001	<0.0001

Bolded row represents all regions combined (overall).
Shaded rows represent individual regions.
*P-value calculated for regional differences without Caribbean values; P-values remained <0.05 when calculated without Canadian or Caribbean values.

and Mid-Atlantic ($n=236$, 22.2%), as compared to the Midwest ($n=66$, 5.7%; $p<0.001$ and $p<0.001$, respectively) and the South ($n=100$, 3.3%; $p<0.001$ and $p<0.001$, respectively). *Aph* seroprevalence was significantly higher in the Northeast ($n=69$, 13%) and the Mid-Atlantic ($n=58$, 5.5%) when compared with other regions in the US ($p<0.01$, all comparisons). *Eew* and *Ech* exposures were most prevalent in Mid-Atlantic ($n=61$, 5.7%; $n=59$, 5.5%, respectively) and Southern dogs ($n=156$, 5.2%; $n=129$, 4.3%, respectively) compared to the Midwest ($n=14$, 1.2%; $n=8$, 0.7%, respectively) ($p<0.001$ for all comparison listed) and did not significantly differ across the Mid-Atlantic and Southern regions. *Ec* prevalence was low among all US and Canadian regions (ranging from 0.5 to 3.2%), with the highest prevalence in the West ($n=12$; 2.4%) and Canada ($n=9$, 3.2%). The Caribbean had a significantly higher *Ec* seroprevalence ($n=8$, 27.6%) than all other regions ($p<0.001$, all comparisons). The *Apl* seroprevalence ranged from a high of 10.3% ($n=3$) in the Caribbean to a low of 0.6% ($n=7$) in the Midwest.

Due to the lack of complete 2012 data for the entire year, significant differences in overall and regional seroprevalences were evaluated by year and month using only data from years 2008 ($n=2{,}327$; 35.4%), 2009 ($n=2{,}184$; 33.2%), and 2010 ($n=1{,}759$; 27%) (Table 2). There were significant differences in the overall *Aph*, *Anaplasma* spp., and *Ech* seroprevalences by year ($p<0.0001$, $p=0.0024$, and $p=0.0004$, respectively). Overall *Ech* exposure appeared to decline from 2008 to 2009, but increased in 2010, while *Aph* increased. Regionally, significant increases in seroprevalence were observed in the Mid-Atlantic, including *Aph* ($p=0.0026$), and the South, including *Aph* and *Bb* ($p<0.0001$ and $p<0.0001$, respectively). The South also had significant changes in *Ech* and *Eew* seroprevalences, with a decline in *Eew* and *Ech* exposure in 2009 followed by an increase in 2010 ($p=0.0191$ and $p=0.0001$, respectively). No significant changes or trends were observed when seroprevalences were compared between months (data not shown). Seroprevalence was determined for each state within the US (Table 1). States with no sample submissions included HI, AK, MT, ID, SD, and ND. Heat maps of the US were generated when in-state seroprevalence data were based upon ≥ 30 submissions (Figs. 1–3).

Co-exposures, defined as seroreactivity to more than one *Anaplasma* spp., *Ehrlichia* spp., or *Bb*, were detected in 261 dogs (4.0%). Seroreactivity to two pathogens occurred in 207 dogs (3.1%); three pathogens in 44 dogs (0.7%); four pathogens in seven dogs (0.1%); and five pathogens in three dogs (0.05%). The most common co-exposures included *Eew*+*Ech* ($n=91$, 1.4%); *Aph*+*Bb* ($n=76$, 1.2%); and *Eew*+*Bb* ($n=41$, 0.6%), in contrast to *Ec*+*Apl* ($n=18$, 0.3%) (Table 3). Notable regional co-exposures included *Aph*+*Bb* in the Northeast ($n=33$; 6.2%), *Eew*+*Ech* in the South ($n=62$; 2.1%) and Mid-Atlantic ($n=22$; 2.1%). The Mid-Atlantic had the highest co-exposure seroprevalence rates for several unexpected pathogen combinations including *Eew*+*Bb* ($n=19$; 1.8%), *Ech*+*Bb* ($n=17$; 1.6%), and *Ech*+*Aph* ($n=12$; 1.1%) (Table 3). ORs identified associations among CVBD co-exposures (Table 3). The highest ORs were found among pathogens known to share a common tick vector (*Eew*+*Ech*: OR = 31.9, 95% CI = 23.2–43.8; *Ec*+*Apl:* OR = 14.3, 95% CI = 8.3–24.8). The OR for *Aph*+*Bb* (OR = 6.2, 95% CI = 4.6–8.3) was lower by comparison. The lower ORs were found among unexpected combinations of pathogens (*Ec*+*Bb*: OR = 0.2, 95% CI = 0.05–0.8; *Apl*+*Bb*: OR = 2.2, 95% CI = 1.3–3.7; and *Ec*+*Ech*: OR = 2, 95% CI = 0.9–4.5) (Table 3).

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Table 2. Seroprevalence per year between 2008 and 2010 in the US, Canada, and Caribbean to *Ehrlichia canis* (*Ec*), *E. ewingii* (*Eew*), *E. chaffeensis* (*Ech*), *Anaplasma platys* (*Apl*), *A. phagocytophilum* (*Aph*), *Borrelia burgdgorferi* (*Bb*), *Anaplasma* spp. (A-genus), and *Ehrlichia* spp. (E-genus)

Years (2008–2010)	Sample #	*Eew* (%)	*Ech* (%)	*Ec* (%)	*Apl* (%)	*Aph* (%)	*Bb* (%)	A-genus (%)	E-genus (%)
Overall									
2008	2,327	91 (3.9)	77 (3.3)	43 (1.8)	40 (1.7)	52 (2.2)	191 (8.2)	76 (3.3)	107 (4.6)
2009	2,184	67 (3.1)	43 (2.0)	36 (1.6)	27 (1.2)	73 (3.3)	165 (7.6)	67 (3.1)	103 (4.7)
2010	1,759	78 (4.4)	72 (4.1)	35 (2.0)	28 (1.6)	96 (5.5)	170 (9.7)	88 (5.0)	103 (5.9)
p		0.073	0.0004	0.721	0.395	<0.0001	0.055	0.0024	0.14
South									
2008	1,030	57 (5.5)	49 (4.8)	29 (2.8)	25 (2.4)	15 (1.5)	24 (2.3)	29 (2.8)	68 (6.6)
2009	1,041	37 (3.6)	23 (2.2)	25 (2.4)	16 (1.5)	14 (1.3)	20 (1.9)	19 (1.8)	62 (6.0)
2010	761	48 (6.3)	47 (6.2)	14 (1.8)	14 (1.8)	32 (4.2)	46 (6.0)	28 (3.7)	68 (8.9)
p		0.0191	0.0001	0.411	0.3309	<0.0001	<0.0001	0.0525	0.0412
Mid-Atlantic									
2008	472	26 (5.5)	24 (5.1)	4 (0.8)	5 (1.1)	14 (3.0)	102 (21.6)	21 (4.4)	25 (5.3)
2009	334	17 (5.1)	15 (4.5)	1 (0.3)	4 (1.2)	24 (7.2)	77 (23.1)	18 (5.4)	18 (5.4)
2010	231	17 (7.4)	20 (8.7)	4 (1.7)	3 (1.3)	20 (8.7)	54 (23.4)	16 (6.9)	16 (6.9)
p		0.4935	0.0817	0.1959	0.9583	0.0026	0.829	0.3863	0.6536
Northeast									
2008	202	3 (1.5)	1 (0.5)	0	6 (3.0)	17 (8.4)	45 (22.3)	17 (8.4)	1 (0.5)
2009	162	7 (4.3)	2 (1.2)	0	2 (1.2)	24 (14.8)	37 (22.8)	15 (9.3)	5 (3.1)
2010	153	8 (5.2)	2 (1.3)	3 (2.0)	0	25 (16.3)	36 (23.5)	19 (12.4)	4 (2.6)
p		0.1	0.6785	n/a	0.0745	0.0551	0.962	0.43	0.1562
Midwest									
2008	409	5 (1.2)	3 (0.73)	3 (0.73)	1 (0.24)	5 (1.2)	16 (3.9)	7 (1.7)	7 (1.7)
2009	382	6 (1.6)	2 (0.52)	0	2 (0.52)	7 (1.8)	26 (6.8)	8 (2.1)	8 (2.1)
2010	322	3 (0.93)	3 (0.93)	2 (0.62)	4 (1.2)	10 (3.1)	23 (7.1)	16 (5.0)	4 (1.2)
p		0.79	0.9	0.28	0.27	0.18	0.11	0.02	0.36
West									
2008	88	0	0	3 (3.4)	1 (1.1)	1 (1.1)	1 (1.1)	1 (1.1)	1 (1.1)
2009	168	0	1 (0.6)	6 (3.6)	1 (0.6)	3 (1.8)	3 (1.8)	1 (0.6)	6 (3.6)
2010	209	2 (0.96)	0	3 (1.4)	3 (1.4)	6 (2.9)	10 (4.8)	4 (1.9)	3 (1.4)
p		n/a	n/a	0.34	0.84	0.59	0.12	0.5242	0.36
Canada									
2008	119	0	0	2 (1.7)	2 (1.7)	0	3 (2.5)	0	3 (2.5)
2009	85	0	0	1 (1.2)	1 (1.2)	1 (1.2)	2 (2.4)	1 (1.2)	1 (1.2)
2010	74	0	0	6 (8.1)	2 (2.7)	2 (2.7)	1 (1.4)	4 (5.4)	5 (6.8)
p		n/a	n/a	0.03	0.73	0.11	0.8532	0.01	0.17
Caribbean									
2008	7	0	0	2 (28.6)	0	0	0	1 (14.3)	2 (28.6)
2009	12	0	0	3 (25.0)	1 (8.3)	0	0	5 (41.7)	3 (25)
2010	9	0	0	3 (33.3)	2 (22.2)	1 (11.1)	0	1 (11.1)	3 (33.3)
p		n/a	n/a	1	0.44	n/a	n/a	0.289	1

Discussion

This study utilized a panel of species-specific, CVBD peptides to determine regional seroprevalences in dogs with suspected tick-borne pathogen exposure. *Ec, Ech, Eew, Aph, Apl,* and *Bb* peptides were designed to detect species-specific antibodies, so as to facilitate identification of unique patterns of CVBD exposure in dog sera from the US, Canada, and the Caribbean (9–12). Significant regional changes and various co-exposure patterns were identified overall, regionally and during 2008–2010; however, significant patterns were not observed between months or seasons (data not shown) of the year, likely because these data do not represent infection onset. Limitations of this study include the following: Sample submission was not proportional across regions with a near majority of specimens submitted from the Southern

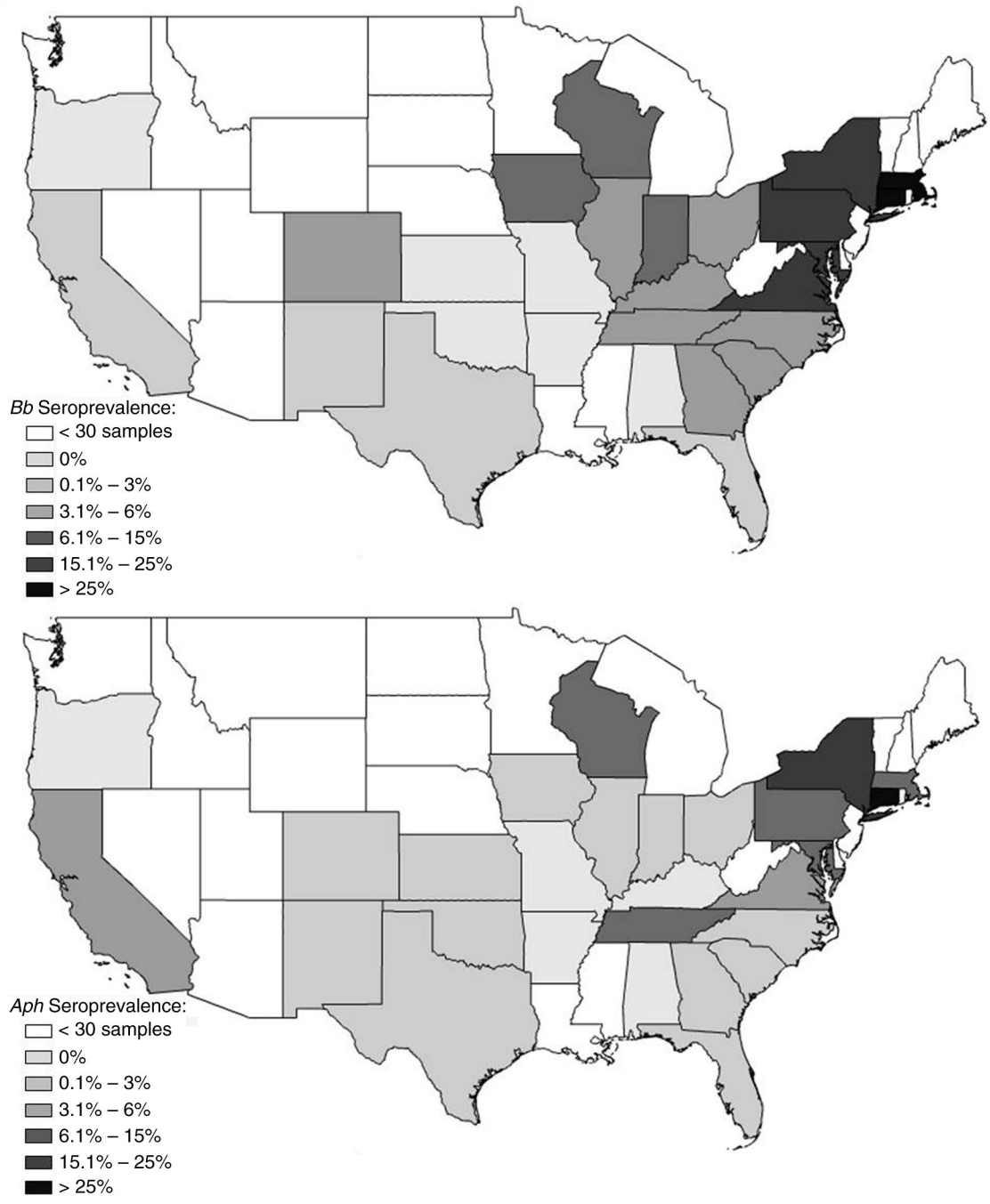

Fig. 1. Seroprevalence by state of *Borrelia burgdorferi* (*Bb*) or *Anaplasma phagocytophilum* (*Aph*) in dogs suspected of canine vector-borne disease.

region (45.7%) compared to the Northeast (8%), the West (7.6%), Canada (4.3%), and the Caribbean (0.4%). Specimens were regionalized based on local veterinary hospital or owner zip codes, and individual dog travel histories were not available. All samples from NCSU-College of Veterinary Medicine were regionalized according to owner zip codes; however, 21% ($n = 1{,}353$) of samples submitted from other veterinary teaching hospitals may not accurately represent local exposure, since clients may travel farther distances for specialized services offered at large teaching hospitals. As this convenience sample was submitted to the VBDDL from dogs suspected of a CVBD, seroprevalence rates are most likely higher than in the general dog population.

Bb (8.3%), the etiologic agent of Lyme disease, was the most seroprevalent pathogen in this convenience sample of dogs ($n = 6{,}582$). This finding is consistent with a recent study involving a large cohort of dogs from the

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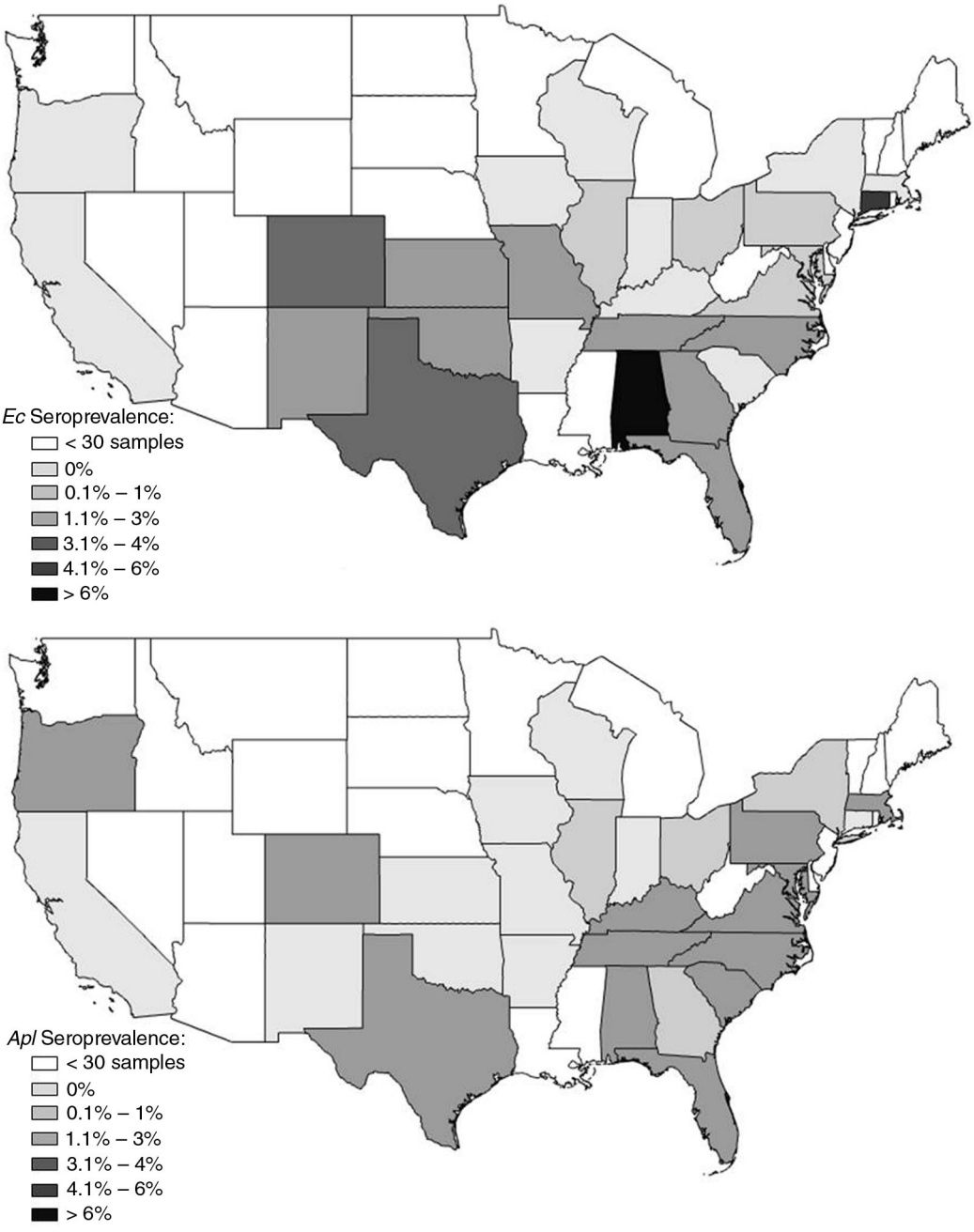

Fig. 2. Seroprevalence by state of *Ehrlichia canis* (*Ec*) or *Anaplasma platys* (*Apl*) in dogs suspected of canine vector-borne disease.

US that reported an overall canine *Bb* seroprevalence, defined as seroreactivity to C_6 peptide, of 7.2% (509,195/6,996,197) (13). This is an increase from an earlier, similar study, which showed an overall canine *Bb* seroprevalence of 5.1% (49,817/982,336) (14). Lyme disease is the most prevalent tick-borne disease in humans in the US and has historically been confined to Northeast and upper Midwestern regions of the country (15, 16). Notably, we documented a statistical increase in *Bb* seroprevalence from 2008 to 2010 in the South ($p < 0.0001$) (Table 2), a region not historically endemic for *Bb* infection. A study by Duncan et al. using a convenience sample from sick dogs submitted for testing to the VBDDL between 2001 and 2003 measured a lower seroprevalence of *Bb*, defined as C_6 seroreactivity, in individual Southern states, including, NC (0.4%), VA (8.7%), and MD (14.4%) than the *Bb* seroprevalences reported in this study (NC, 5.4%; VA, 20.3%; MD, 24.9%) (8). Notably, the seroprevalence of *Bb* in northern states was more similar between the two studies (25% vs. 22.2%, respectively, in PA) suggesting the differences in the South are more likely due to prevalence changes and less likely testing variations. Our study

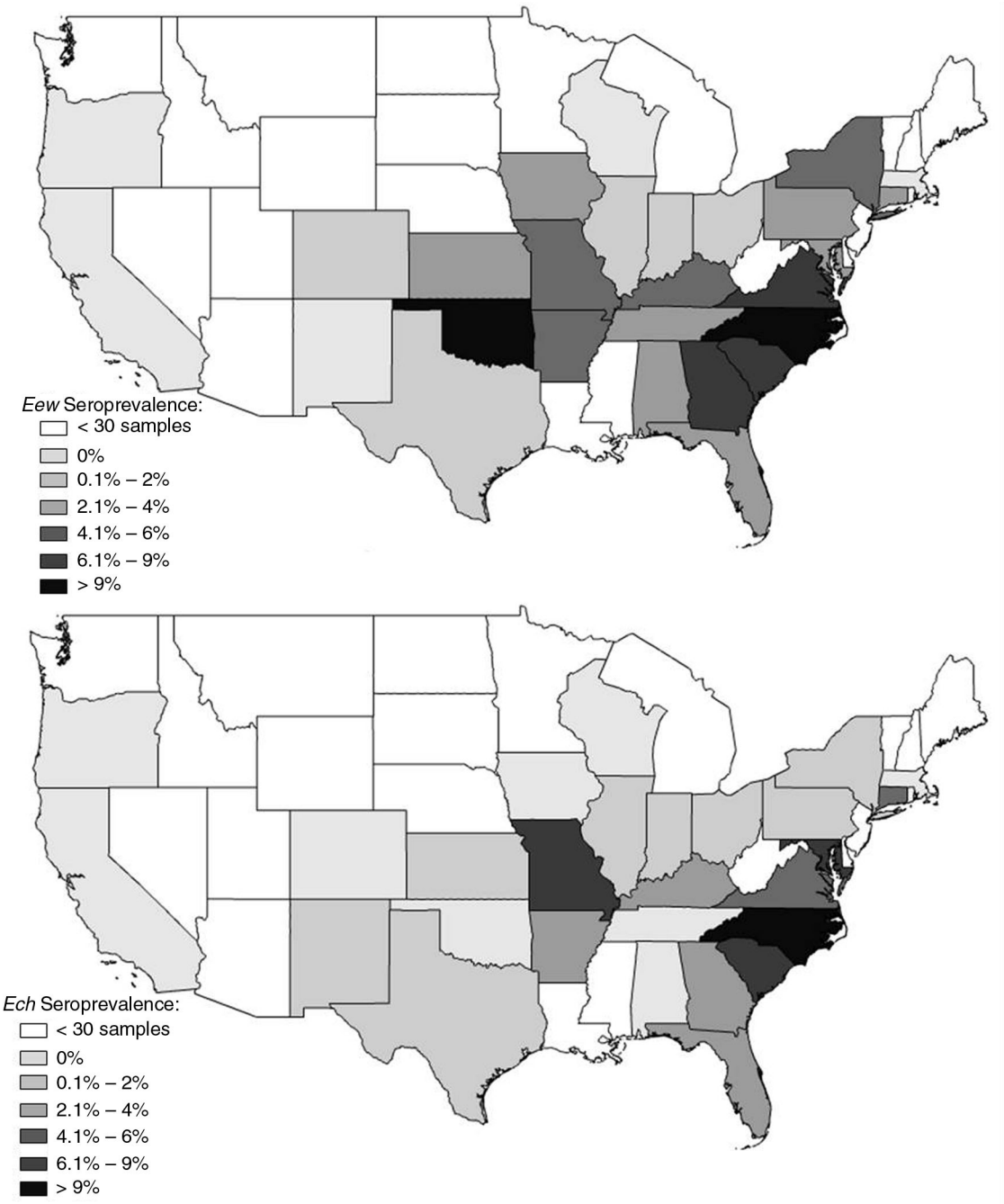

Fig. 3. Seroprevalence by state of *Ehrlichia ewingii* (*Eew*) or *E. chaffeensis (Ech*) in dogs suspected of canine vector-borne disease.

found the *Bb* seroprevalence in dogs from the Mid-Atlantic ($n = 236$; 22.2%), a region bordering the South, to approximate the *Bb* seroprevalence in the Northeast ($n = 122$; 22.9%) ($p = 0.99$). Furthermore, one third ($n = 1{,}014$; 34%) of the samples from the South in this study were collected from dogs residing in NC, a state that borders VA, where according to the CDC, an increase in Lyme disease incidence had been reported in recent years (17). Recently, VA established five counties along the NC border endemic for Lyme disease (18, 19). The increased *Bb* seroprevalence observed in dogs from the Southern US supports a potential trend for *Bb* expansion southward, warranting further studies to monitor Lyme disease in both dogs and humans south of Mid-Atlantic States. The CDC reports the approximate distribution of *I. scapularis* extends from Texas to the Southeast, Mid-Atlantic, Northeast, and upper Midwestern states, and a recent report has documented population increases in Canada (20, 21). We found a *Bb* seroprevalence of 2.1% ($n = 6$) within our Canadian dog

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Table 3. Co-exposures from all samples collected between 2008–2010 and 2012 ($n = 6{,}582$) with corresponding seroprevalence (%) and odds ratios (OR) with 95% confidence interval (CI) to *Ehrlichia canis* (*Ec*), *E. ewingii* (*Eew*), *E. chaffeensis* (*Ech*), *Anaplasma platys* (*Apl*), *A. phagocytophilum* (*Aph*), and *Borrelia burgdgorferi* (*Bb*)

Co-exposure	[a]Overall	South	Mid-Atlantic	Northeast	Midwest	West	Canada	Caribbean
	$n = 6{,}582$	$n = 3{,}011$	$n = 1{,}065$	$n = 532$	$n = 1{,}162$	$n = 498$	$n = 285$	$n = 29$
Ew+*Ech*	91 (1.4) OR = 31.9 95% CI 23.2–43.8	62 (2.1)	22 (2.1)	4 (0.8)	3 (0.3)	0	0	0
Aph+*Bb*	76 (1.2) OR = 6.2 95% CI 4.6–8.3	17 (0.6)	19 (1.8)	33 (6.2)	5 (0.4)	2 (0.4)	0	0
Ew+*Bb*	41 (0.6) OR = 2.3 95% CI 1.6–3.2	19 (0.6)	19 (1.8)	3 (0.6)	0	0	0	0
Ech+*Bb*	36 (0.5) OR = 2.5 95% CI 1.7–6.3	17 (0.6)	17 (1.6)	2 (0.4)	0	0	0	0
Ech+*Aph*	29 (0.4) OR = 5.2 95% CI 3.4–8.0	14 (0.5)	12 (1.1)	3 (0.6)	0	0	0	0
Aph+*Apl*	25 (0.4) OR = 9.9 95% CI 6.1–16	11 (0.4)	4 (0.4)	6 (1.1)	1	1	1	1
Ew+*Aph*	24 (0.4) OR = 3.2 95% CI 2.0–5.0	11 (0.4)	6 (0.6)	6 (1.1)	0	1	0	0
Ec+*Apl*	18 (0.3) OR = 14.3 95% CI 8.3–24.8	10 (0.3)	1 (0.09)	0	0	3 (0.6)	3 (1.0)	1
Apl+*Bb*	17 (0.3) OR = 2.2 95% CI 1.3–3.7	7 (0.2)	4 (0.4)	5 (0.9)	1	0	0	0
Apl+*Ew*	13 (0.2) OR = 4 95% CI 2.2–7.2	5 (0.2)	4 (0.4)	2 (0.4)	1	1	0	0
Apl+*Ech*	13 (0.2) OR = 5 95% CI 2.9–9.2	9 (0.3)	2 (0.2)	1	1	0	0	0
Ec+*Aph*	12 (0.2) OR = 3.3 95% CI 1.8–6.1	5 (0.2)	2 (0.2)	1	1	0	2 (0.7)	1
Ec+*Ew*	10 (0.2) OR = 2.4 95% CI 1.3–4.7	9 (0.3)	1	0	0	0	0	0
Ec+*Ech*	7 (0.1) OR = 2 95% CI 0.9–4.5	6 (0.2)	0	0	0	1	0	0
Ec + *Bb*	2 (0.03) OR = 0.2 95% CI 0.05–0.8	0	1	1	0	0	0	0

[a]OR and 95% CI calculated for overall co-exposures only.

population ($n = 285$). Previous studies measuring canine seroreactivity to C_6 peptide reported lower *Bb* seroprevalences in Canada; canine sera collected from southern Ontario and Quebec between 2000 and 2003 ($n = 108$) reported *Bb* seroprevalence as 1.85%, while another study in 2008 that included all provinces found an overall seroprevalence of 0.72% ($n = 624$) (22, 23). The increased seroprevalence could be related to differences in testing platforms, health status of the dogs, population number and distribution differences and possibly a northern movement of *Bb* infected ticks. In 2009, Lyme disease became a nationally reportable disease in Canada, with reports of increasing incidence in people (24, 25). Interestingly, a study in dogs using SNAP® 3Dx® and 4Dx® showed the incidence of Lyme in dogs from ON in 2006 (0.36) and 2007 (0.58) is approximate to the incidence reported in people from ON in 2006 (0.35) and 2007 (0.58) (25, 26). These data further support the use of dogs as sentinels for *Bb* exposure in people.

This study documented a significant increase in canine exposure to *Aph* in the US from 2008 to 2010 ($p < 0.0001$) (Table 2), suggesting a progressively increased risk for human *Aph* exposure. These data are supported by the substantial (53%) increase of reported human granulocytic anaplasmosis cases described by the CDC from 2009 to 2010 (27–29). Furthermore, canine *Aph* seroprevalences were high in the Northeast ($n = 69$; 13%), Mid-Atlantic ($n = 58$; 5.4%) and the Midwestern state, WI ($n = 6$; 10.3%) emphasizing the potential utility of dog data for establishing real-time regional human *Aph* exposure risk. The South had a higher *Aph* seroprevalence (2.1%) than previous reports that documented *Anaplasma* spp. ($n = 496$; 0.5% and $n = 1{,}631{,}332$; 0.9%) (13, 14); the discrepancy, in part, could be due to a greater number of sick dogs in this sample set, while the former studies included a larger population of healthy dogs. We identified a significant increase in *Aph* seroprevalence from 2008 to 2010 in the Mid-Atlantic ($p < 0.0001$) and the South ($p < 0.0001$), consistent with *Bb* seroprevalence trends for the Southern region. Like *Bb*, *Aph* is not endemic in the South. Studies reporting the molecular presence of *Aph* in ticks from the South found *Aph* DNA in 1.3% of *I. scapularis* ticks and 2.7% of *A. americanum* ticks collected from rodents in Florida (30); another study found 1.6% *Aph* DNA in *I. scapularis* ticks collected in SC, GA and FL, with the highest prevalence (20%) identified in ticks collected along the GA coast, a documented avian flyway (31).

Despite similar *Aph* and *Bb* seroprevalence trends and a significant *Aph* + *Bb* co-exposure pathogen association (OR = 6.2; 95% CI = 4.6–8.3), overall the *Aph* (3.5%) and *Bb* (8.3%) seroprevalences differed significantly ($p \leq 0.001$). Correspondingly, the prevalence of *Aph* DNA in *I. scapularis* ticks collected in NJ was much lower than *Bb* (6.1% ($n = 9$) and 50.3% ($n = 74$), respectively) (32). In this study, *Bb* seroprevalence was found to be similar among dogs from the Mid-Atlantic (22.2%) and the Northeast (22.9%) ($p = 0.99$); however, the *Aph* seroprevalence differed significantly between the two regions (Mid-Atlantic; 5.4% ($n = 58$) and Northeast; 13% ($n = 69$) ($p \leq 0.001$), potentially reflecting a less prevalent *Aph* infection of ticks in the Mid-Atlantic when compared to the Northeast.

We identified *Eew* (3.8%) as the most common *Ehrlichia* exposure in dogs, followed by *Ech* (3.1%) and *Ec* (1.8%), which is consistent with a 2010 study that found *Eew* (5.1%) as the most seroprevalent *Ehrlichia* spp. pathogen in a large population of dogs from North America ($n = 8{,}622$), when compared to *Ech* (2.8%) and *Ec* (0.8%) (9). A similar study in dogs from the south central US ($n = 143$) detected much higher *Eew* (44.8%) and *Ech* (17.5%) seroprevalences and a similar *Ec* (1.4%) seroprevalence (33). In this study, overall *Ech* seroprevalence varied significantly over time with an initial decrease and then increase in 2010; seroprevalence rates were determined to be 3.3% ($n = 77$) in 2008, 2.0% ($n = 43$) in 2009, and 4.1% ($n = 72$) in 2010 ($p = 0.0004$) (Table 2). This pattern was observed in three regions, the South, Mid-Atlantic and Midwest, in which *A. americanum* ticks are prevalent. Reported *Ech* human monocytic ehrlichiosis (HME) cases increased before a significant drop in 2010 (27–29), which did not mirror our canine *Ech* seroprevalence. Regionally, however, the high prevalence of HME cases was largely similar to dog *Ech* seroprevalence, with highest exposure risk in the South and Mid-Atlantic (27–29). In the South, canine *Eew* seroprevalence also showed statistically significant changes over 2008 ($n = 57$; 5.5%), 2009 ($n = 37$; 3.6%), and 2010 ($n = 48$; 6.3%) ($p = 0.02$) (Table 2), which mirrored the trend for human *Eew* cases (*Eew* ehrlichiosis) for 2008–2010 (27–29). The difficulty in clinically distinguishing between HME and *Eew* ehrlichiosis, along with the low number of human *Eew* infection reports could complicate comparisons made between canine and human *Eew* exposure (5, 28); nevertheless, reports of high canine *Eew* seroprevalences should prompt more consideration for greater *Eew* exposure risk in humans throughout much of the Central and Southern US. In 2008, CDC made *Eew* ehrlichiosis a reportable disease in humans (29).

Overall, *Ec* ($n = 117$; 1.8%) and *Apl* ($n = 99$; 1.5%) had the lowest seroprevalences in dogs from the US. Exposure frequencies were high in the Caribbean ($n = 8$; 27.6% and $n = 3$; 10.3%, respectively), as expected, where *R. sanguineus,* the known vector for *Ec* and potential vector for *Apl,* is prevalent. A previous study reported high *Ec* sero- (43.8%) and PCR (24.7%) prevalences in the Caribbean (4). *R. sanguineus* is rarely documented in Canada (34); however, Canada had unexpectedly high *Ec* and *Apl* seroprevalences ($n = 9$; 3.2% and $n = 5$; 1.8%, respectively), potentially due to a reporting bias from low numbers tested in this study or

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because dogs had traveled to or were transported from *Ec* and *Apl* endemic regions. Efforts to relocate homeless animals, particularly from tropical regions, including the Caribbean, to the Northeastern US and Canada have increased. For example, in 2003 the Save a Sato Foundation, which aims to relocate homeless dogs in Puerto Rico to the US, transported roughly 14,000 dogs to the US (35). Relocating animals to shelter environments in non-endemic US regions and Canada could create *R. sanguineus* infestations within kennels, significantly impacting the prevalence rates of foreign tick-borne pathogen strains within the local dog population and exposing people to foreign, zoonotic pathogens.

Co-infections complicate interpretation of the clinical manifestations typically associated with single tick-borne diseases in both canine and human medicine. Co-infections can occur from simultaneous or sequential exposure to several tick species, or when multiple pathogens are transmitted by a single tick (2, 3, 36). In our study, co-exposures were defined as dogs seropositive to two or more vector-borne pathogens. Overall, the co-exposure seroprevalence rates were low. Combinations with the highest seroprevalence rates were among pathogens known to share a common tick vector such as *Eew* + *Ech* in *A. americanum* and *Aph* + *Bb* in *I. scapularis*. Regional co-exposure seroprevalences were highest in areas where the respective shared tick species are endemic, including *Eew* + *Ech* in the South and Mid-Atlantic, and *Aph* + *Bb* in the Northeast. Interestingly, the Mid-Atlantic had the highest co-exposure seroprevalence rates for several unexpected pathogen combinations including *Eew* + *Bb*, *Ech* + *Bb*, and *Ech* + *Aph* (Table 3). These co-exposure combinations and seroprevalence rates highlight the Mid-Atlantic as a potential region where *I. scapularis* and *A. americanum* ticks and their respective pathogens coalesce. As tick species migrate and habitats overlap, co-exposures will likely be more common with the potential for more disease severity. When monitoring tick-borne diseases in regions like the Mid-Atlantic, co-infections should be considered.

In conclusion, this study provides further support for the use of dogs in tick-borne pathogen human surveillance risks for several zoonotic infections of human and veterinary medical importance. Over a relatively brief time period, we demonstrated significant shifts in CVBD seroprevalence rates including overall increases in *Aph* and *Ech*, increases in *Aph* in the Mid-Atlantic and the South and increases in *Bb* in the South. Furthermore, by recognized species-specific seroprevalence, expected and unique co-exposures were identified and highlight the potential for tick-borne pathogen co-infections. Combining dog and human tick-borne disease surveillance data could enhance both public health and animal health.

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