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

Citation: Infection Ecology and Epidemiology 2014, 4: 24699 - http://dx.doi.org/10.3402/iee.v4.24699

(Received: 18 April 2014; Revised: 11 September 2014; Accepted: 19 September 2014; Published: 20 October 2014)

Cine 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
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 pep-
tides developed to detect canine antibodies to Ec, Ech,
Eew, Aph, and Apl were used to manufacture a pro-
prietary, 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 Labora-
tory 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 infor-
mation 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 com-
bination 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, sequen-
tial flow of wash solution and enzyme substrate. Archived
canine serum stored at −80°C was thawed to room tem-
perature 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 Multitests procedure in SAS/STAT v.9.3 (SAS
Institute, Cary, NC). Regions were assigned into the fol-
lowing 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 sero-
positive 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 patho-
gen (representing concurrent or sequential co-exposures).
Level of significance was established at p < 0.05. Sta-
tistical analyses were performed using SAS/STAT 9.3 (SAS
Institute Inc., Cary, NC).

Results

A total of 6,582 dog serum samples 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 docu-
mented 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, re-
spectively, 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%)
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 burgdorferi* (*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     | 202     | 117     | 99      | 227     | 545    | 238         | 327         |
| South         | 3,011    | 156     | 129     | 70      | 59      | 64      | 100    | 80          | 209         |
| FL            | 501      | 15      | 11      | 13      | 12      | 10      | 8      | 19          | 21          |
| GA            | 162      | 12      | 6       | 2       | 1       | 1       | 6      | 3           | 6           |
| NC            | 1,014    | 104     | 96      | 19      | 22      | 25      | 55     | 26          | 120         |
| SC            | 93       | 8       | 7       | 0       | 1       | 1       | 5      | 2           | 7           |
| AL            | 40       | 1       | 2       | 0       | 1       | 2       | 0      | 0           | 2           |
| AR            | 36       | 2       | 1       | 0       | 0       | 0       | 0      | 0           | 2           |
| KY            | 69       | 3       | 2       | 0       | 1       | 1       | 4      | 1           | 5           |
| LA            | 27       | 1       | 1       | 1       | 0       | 0       | 0      | 1           | 3           |
| MS            | 16       | 0       | 0       | 1       | 0       | 0       | 0      | 0           | 1           |
| OK            | 42       | 6       | 1       | 0       | 1       | 1       | 0      | 1           | 4           |
| TN            | 45       | 1       | 1       | 1       | 2       | 2       | 4      | 0           | 4           |
| TX            | 966      | 3       | 5       | 30      | 19      | 21      | 20     | 27          | 36          |
| Mid-Atlantic  | 1,065    | 61      | 59      | 9       | 12      | 58      | 236    | 55          | 60          |
| VA            | 656      | 45      | 32      | 6       | 7       | 28      | 133    | 30          | 38          |
| MD            | 313      | 12      | 25      | 4       | 25      | 78      | 24     | 21          | 20          |
| DE            | 4        | 0       | 0       | 0       | 0       | 1       | 25     | 0           | 0           |
| DC            | 90       | 4       | 2       | 0       | 1       | 5       | 24     | 4           | 2           |
| WV            | 2        | 0       | 0       | 0       | 0       | 0       | 0      | 0           | 0           |
| Northeast     | 532      | 18      | 5       | 3       | 8       | 69      | 122    | 54          | 10          |
| CT            | 48       | 1       | 2       | 2       | 0       | 12      | 16     | 11          | 3           |
| MA            | 35       | 0       | 0       | 1       | 2       | 5       | 14     | 4           | 0           |
| ME            | 4        | 0       | 0       | 0       | 1       | 0       | 1      | 0           | 0           |
| NH            | 19       | 0       | 0       | 0       | 0       | 1       | 5      | 1           | 5           |
| VT            | 6        | 0       | 0       | 0       | 0       | 2       | 3      | 1           | 0           |
| NJ            | 12       | 0       | 0       | 0       | 0       | 1       | 8      | 1           | 8           |
| NY            | 205      | 10      | 2       | 1       | 0       | 1       | 33     | 35          | 24          |
| PA            | 203      | 7       | 1       | 0       | 1       | 5       | 15     | 45          | 12          |
| Midwest       | 1,162    | 14      | 8       | 6       | 7       | 22      | 66     | 31          | 20          |
| MI            | 20       | 0       | 0       | 0       | 0       | 0       | 0      | 1           | 5           |
| OH            | 430      | 2       | 1       | 1       | 3       | 3       | 16     | 7           | 5           |
| IN            | 93       | 1       | 1       | 0       | 0       | 2       | 9      | 3           | 2           |
| IL            | 383      | 4       | 2       | 1       | 4       | 7       | 23     | 12          | 5           |
| WI            | 58       | 0       | 0       | 0       | 0       | 6       | 12     | 5           | 0           |
| MN            | 8        | 0       | 1       | 12      | 0       | 2       | 25     | 12          | 0           |
| IA            | 78       | 3       | 0       | 0       | 0       | 1       | 9      | 2           | 1           |
| MO            | 36       | 2       | 3       | 1       | 0       | 0       | 0      | 4           | 11          |
| KS            | 53       | 2       | 1       | 1       | 0       | 0      | 0      | 1           | 1           |
| NE            | 3        | 0       | 0       | 1       | 3       | 0       | 0      | 1           | 3           |
| West          | 498      | 2       | 1       | 12      | 5       | 10      | 15     | 6           | 10          |
| AZ            | 15       | 1       | 0       | 0       | 0       | 1       | 6      | 0           | 0           |
| CA            | 121      | 0       | 0       | 0       | 0       | 4       | 3      | 1           | 0           |
| CO            | 246      | 1       | 9       | 3       | 5       | 10      | 4      | 4           | 8           |
| NM            | 61       | 0       | 1       | 1       | 0       | 1      | 1      | 0           | 1           |
| NV            | 5        | 0       | 0       | 0       | 0       | 0      | 0      | 0           | 0           |
| OR            | 35       | 0       | 0       | 0       | 1      | 0      | 0      | 0           | 0           |
| UT            | 2        | 0       | 0       | 0       | 1       | 0      | 0      | 0           | 1           |
| WA            | 12       | 0       | 0       | 0       | 0       | 0      | 0      | 0           | 0           |
| WY            | 1        | 0       | 0       | 1       | 1       | 0      | 0      | 1           | 0           |

Citation: Infection Ecology and Epidemiology 2014, 4: 24699 - http://dx.doi.org/10.3402/iee.v4.24699
and Mid-Atlantic (n=236, 22.2%), as compared to the Midwest (n=66, 5.7%; p < 0.0001 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 *Ec + Apl* (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).
This study utilized a panel of species-specific, CVBD peptides to determine regional seroprevalences in dogs with suspected tick-borne pathogen exposure. *Ehrlichia canis* (Ec), *E. chaffeensis* (Ech), *Anaplasma platys* (Apl), *A. phagocytophilum* (Aph), *Borrelia burgdorferi* (Bb), *Anaplasma* spp. (A-genus), and *Ehrlichia* spp. (E-genus) 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

### 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 burgdorferi* (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                 | 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     | 1 (14.3)    | 2 (28.6)    |
|                   | 2009      | 12       | 0       | 0       | 3 (25.0)| 1 (8.3)| 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     | 0.289  | 1     |             |             |
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
US that reported an overall canine *Bb* seroprevalence, defined as seroreactivity to C6 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 C6 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. 2. Seroprevalence by state of *Ehrlichia canis* (*Ec*) or *Anaplasma platys* (*Apl*) in dogs suspected of canine vector-borne disease.](image-url)
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
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 burgdorferi* (Bb)

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

*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
because dogs had traveled to or were transported from Ec and Ap/ 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.

Acknowledgements

We thank Tonya Lee for editorial assistance. We thank the Vector-borne Disease and Diagnostic Laboratory at North Carolina State University, Raleigh, NC, for access to archived canine serum.

Conflict of interest and funding

The authors have not received any funding or benefits from industry or elsewhere to conduct this study.

Disclaimer

Barbara Qurollo's fellowship in Vector-Borne Disease Research at the College of Veterinary Medicine, North Carolina State University is supported by IDEXX Laboratories. Ramaswamy Chandrashekar, Melissa J. Beall, Brett A. Stillman, Jiayou Liu, and Brendon Thatcher are employees of IDEXX Laboratories, and Edward B. Breitschwerdt is a consultant to the company in the area of tick-borne infectious diseases.

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Citation: Infection Ecology and Epidemiology 2014, 4: 24699 - http://dx.doi.org/10.3402/iee.v4.24699