Multitarget Test for Emerging Lyme Disease and Anaplasmosis in a Serosurvey of Dogs, Maine, USA

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To determine if the range of deer ticks in Maine had expanded, we conducted a multtarget serosurvey of domestic dogs (Canis lupus familiaris) in 2007. An extension of exposure to Borrelia burgdorferi to the northern border and local transmission of Anaplasma phagocytophilum throughout southern areas was found.

Over the past 2 decades, the range of Ixodes scapularis, the deer tick, vector of Lyme disease, anaplasmosis, babesiosis, and deer tick virus infections, has expanded in northern New England. Because Lyme disease and anaplasmosis affect humans and dogs (Canis lupus familiaris), serosurveys of canids have proved useful for monitoring emergence of these infections. Sample selection may be confounded when dogs that are remotely exposed, vaccinated, or treated with topical acaricides are included. In recent years, however, the advent of a multitarget, in-clinic test kit (SNAP 4Dx; IDEXX Laboratories, Westbrook, ME, USA) has increased the scope and efficiency of these serosurveys. The SNAP4Dx tests for heartworm antigen and antibodies to Borrelia burgdorferi, Anaplasma phagocytophilum, and Ehrlichia canis on 3 drops of blood. Its sensitivity and specificity for antibodies against B. burgdorferi and A. phagocytophilum exceed 98% (1,2).

In Maine, deer ticks were first reported at a coastal site in 1988 and have since spread inland (3). Lyme disease has become a major public health problem; reported human cases reached 169 per 100,000 population in 1 mid-coastal county in 2008. Human cases of anaplasmosis and babesiosis are also being reported (4). In 1990, we conducted a statewide serosurvey to map B. burgdorferi–positive dogs and to correlate their distribution with reported human cases. Four percent of 828 samples were seropositive for B. burgdorferi, 89% of which were from dogs residing within 20 miles of the coast. No positivity was found among 102 dogs in the northern half of the state (5). Given the widespread acceptance of SNAP 4Dx tests by Maine veterinarians, we resurveyed dogs statewide in 2007 for exposure to B. burgdorferi and A. phagocytophilum. Data from questionnaires to veterinarians and dog owners enabled assessment of the influence of the use of Lyme vaccines and topical acaricides on canine serologic test results.

The Study

From 87 veterinary clinics solicited in 2007, we selected 47 on the basis of their size and geographic distribution. Each was supplied with 15–30 SNAP 4Dx kits (contributed by IDEXX Laboratories). Veterinarians were instructed to obtain samples from all dogs routinely tested for heartworm. In northern areas, where heartworm is rarely tested for, they were asked to collect samples from dogs undergoing surgery. They recorded each dog’s age, town of residence, Lyme disease vaccination status (ever or never vaccinated), and the test results. Each dog owner completed a form (99.6% response rate) to describe the dog, its function, history of unexplained lameness, travel history (town, state, visited within the past year), history of tick infestation, and use of tick control products (yes or no).

We summarized test results to town and county levels. We used Spearman rank correlation tests to examine associations between canine seropositivity, human Lyme disease cases reported for 2007 (4), and the number of deer ticks submitted to our laboratory in 2007. We used B. burgdorferi and A. phagocytophilum test results and questionnaire responses to cross-tabulate responses and calculate the likelihood (odds ratios) of B. burgdorferi and A. phagocytophilum positivity as a function of risk factors by using 2 tests of association. We considered differences significant at p<0.05. Analyses were conducted by using SAS version 9.2 for Windows (SAS, Cary, NC, USA).

Of 1,087 dogs tested across Maine’s 16 counties, 12.7% were B. burgdorferi–positive and 7.1% were A. phagocytophilum–positive (Table 1); 1.9% were co-infected. The distribution of all dogs seropositive for either pathogen is shown by town in Figure 1. At the county level, canine B. burgdorferi seropositivity among unvaccinated dogs correlated positively with the number of human Lyme disease cases reported for 2007 (p = 0.009) and the number of deer ticks submitted to our laboratory for identification (p = 0.009). In Figure 2, which shows statewide distributions by county north to south, only unvaccinated dogs are included in B. burgdorferi–positive data shown. Dogs had been exposed...
to *A. phagocytophilum* in all but 2 northern counties. At the town level, remarkably higher levels of canine *A. phagocytophilum* seropositivity were found in southern coastal Cape Elizabeth (Cumberland County) (76.5%, n = 17) and York (York County) (58.0%, n = 19) than in towns in their immediate vicinity.

Overall, 54.3% of the dogs had been vaccinated against Lyme disease. Never-vaccinated dogs were 1.5× as likely to be seropositive for *B. burgdorferi* than were vaccinated dogs (15.3% vs. 9.9%; *p* = 0.008) (Table 2). Vaccine use was higher in 10 southern counties where Lyme disease has become endemic (Figure 2, panel B) than in the 6

![Figure 1. Towns where dogs were tested for seropositivity to *Borrelia burgdorferi* (A) and *Anaplasma phagocytophilum* (B) in a statewide serosurvey of domestic dogs, Maine, USA, 2007.](image-url)
northern counties where it is emerging (63.9% vs. 42.7%; 
$p<0.0001$) and correlated positively with the number of 
deer ticks submitted to our laboratory for identification in 
2007 ($n = 16$ counties, $\rho_{\text{Spearman}} = 0.63; p = 0.009$). Two 
thirds of respondents said that their dogs had traveled out 
of town; however, no associations were found between 
$B. burgdorferi$ or $A. phagocytophilum$ seropositivity and the 
dog’s travel history. Three of 59 dogs in the northernmost 
county of Maine were $B. burgdorferi$–positive, 1 of which 
had never traveled beyond its home town. Eighty-three 
percent of owners reported using acaricides. Despite the 
effective protection reported for topical acaricides ($6,7$), no 
difference in seropositivity between treated and untreated 
dogs was evident on the basis of their reported use (Table 
2). Unexplained lameness was $1.5 \times$ more likely in dogs 
that were only $A. phagocytophilum$–positive than in those 
only $B. burgdorferi$–positive (40.0% vs. 26.5%; $p<0.06$).

**Conclusions**

This study demonstrates that risk of contracting 
Lyme disease has reached northernmost Maine and that 
anaplasmosis is now being transmitted to dogs throughout 
the lower half of the state. The study expands on 
nationwide SNAP 4Dx data documenting $B. burgdorferi$ 
and $A. phagocytophilum$ positivity in the southern half 
of the state ($8$). In southern coastal Maine, overabundant 
white-tail deer, appropriate habitat, and maritime climate 
all contribute to high densities of $I. scapularis$ ticks ($3$) 
and consequent disease transmission; thus, the remarkably 
high level of $A. phagocytophilum$ seroreactivity observed 
in the southern coastal towns of Cape Elizabeth and York 
calls for further work to understand the dynamics of the 
intense local emergence of this pathogen. The higher level 
of unexplained lameness in $A. phagocytophilum$–positive 
dogs than in $B. burgdorferi$–positive dogs is consistent with 
findings by Beall et al. ($9$), who reported a $2.6 \times$ greater 
incidence of $A. phagocytophilum$ seroreactivity than $B. 
burgdorferi$ seroreactivity among 32 lame, non–co-infected 
dogs in Minnesota who were suspected of having either 
disease. The lameness also reflects the high percentage of 
$B. burgdorferi$ positivity among asymptomatic dogs ($10$). 
That $B. burgdorferi$ and $A. phagocytophilum$ seropositivity 
rates were essentially identical between dogs who had a 
history of travel and those who did not lessens concern 
about travel as a confounding variable, an exposure dif 
ficult to interpret in any event, given the spotty distribution of 
ticks even where Lyme disease is endemic ($11$).

In a recent study, Hamer et al. ($12$) reported that a 
serosurvey of canines for $B. burgdorferi$ is ineffective 
in a region that includes areas with little $B. burgdorferi$ 
transmission, and less informative than analysis of 
ticks removed from dogs. The authors referred to the 
confounding influence of tick chemoprophylaxis. Our 
inability to detect an effect of topical acaricides may 
reflect their ubiquitous use for flea control and a lack of 
information on the frequency of their use. Although the 
widespread use of protective measures now complicates 
interpretation of serosurveys of canines, in selected dogs 
the availability of a reliable, multitarget test that is used 
routinely nationwide ($8$) remains a valuable and cost-
effective method for documenting transmission of the 
agents of Lyme borreliosis and anaplasmosis, particularly 
in areas where disease is emerging.

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**Figure 2. A)** Canine seroprevalence for *Anaplasma phagocytophilum* 
and, in dogs never vaccinated against Lyme disease, for *Borrelia 
burgdorferi* in Maine counties arranged north to south, 2007. **B)** 
Maine counties, with the 10 tick-abundant counties used in the 
analyses shaded in gray. Counties: 1, Aroostook; 2, Piscataquis; 3, 
Somerset; 4, Penobscot; 5, Franklin; 6, Washington; 7, Hancock; 
8, Oxford; 9, Waldo; 10, Kennebec; 11, Knox; 12, Lincoln; 13, 
Androscoggin; 14, Sagadahoc; 15, Cumberland; 16, York.
Table 2. Risk factors vs. canine seroprevalence of *Anaplasma phagocytophilum* and *Borrelia burgdorferi*, Maine, USA, 2007*

| Variable                           | No. dogs | Borrelia burgdorferi No. (%) positive | OR (95% CI) | p value† | Anaplasma phagocytophilum No. (%) positive | OR (95% CI) | p value† |
|-----------------------------------|----------|--------------------------------------|-------------|----------|------------------------------------------|-------------|----------|
| Lyme vaccine                      |          |                                      |             |          |                                          |             |          |
| Yes                               | 575      | 57 (9.9)                             | 1.5 (1.1–2.1) | 0.008    | 49 (8.5)                                |             |          |
| No                                | 483      | 75 (15.3)                            | 23 (4.8)    | 0.6 (0.3–0.9) | 0.02        |
| Travel‡                           |          |                                      |             |          |                                          |             |          |
| All dogs                          |          |                                      |             |          |                                          |             |          |
| None                              | 357      | 49 (13.7)                            | 27 (7.6)    |          |                                          |             |          |
| ≥1                                | 730      | 89 (12.2)                            | 50 (6.9)    | 0.9 (0.6–1.5) | NS          |
| Unvaccinated dogs                 |          |                                      |             |          |                                          |             |          |
| None                              | 163      | 21 (12.9)                            | 8 (4.9)     |          |                                          |             |          |
| ≥1                                | 320      | 53 (16.6)                            | 15 (4.7)    | 0.9 (0.4–2.3) | NS          |
| Tick control products             |          |                                      |             |          |                                          |             |          |
| All dogs                          |          |                                      |             |          |                                          |             |          |
| No                                | 182      | 20 (11.0)                            | 7 (3.9)     |          |                                          |             |          |
| Yes                               | 899      | 115 (12.9)                           | 66 (7.4)    | 2.0 (0.9–4.4) | 0.08        |
| Unvaccinated dogs                 |          |                                      |             |          |                                          |             |          |
| No                                | 124      | 13 (10.5)                            | 3 (2.4)     |          |                                          |             |          |
| Yes                               | 350      | 59 (16.9)                            | 18 (5.1)    | 2.2 (0.6–7.6) | NS          |
| History of unexplained lameness   |          |                                      |             |          |                                          |             |          |
| No                                | 877      | 97 (11.1)                            | 42 (4.8)    |          |                                          |             |          |
| Yes                               | 191      | 140 (29.0)                           | 28 (15.2)   | 3.6 (2.1–5.9) | <0.0001    |

*A total of 1,087 dogs were tested. OR, odds ratio; CI, confidence interval; NS, not significant.

†Significance based on Pearson χ² statistic with 1 degree of freedom.

‡Trips away from town of residence.

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