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Prevalence of Human-Active and Variant 1 Strains of the Tick-Borne Pathogen *Anaplasma phagocytophilum* in Hosts and Forests of Eastern North America

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Abstract. Anaplasmosis is an emerging infectious disease caused by infection with the bacterium *Anaplasma phagocytophilum*. In the eastern United States, *A. phagocytophilum* is transmitted to hosts through the bite of the blacklegged tick, *Ixodes scapularis*. We determined the realized reservoir competence of 14 species of common vertebrate hosts for ticks by establishing the probability that each species transmits two important strains of *A. phagocytophilum* (*A. phagocytophilum* human-active, which causes human cases, and *A. phagocytophilum* variant 1, which does not) to feeding larval ticks. We also sampled questing nymphal ticks from ~150 sites in a single county over 2 years and sampled over 6 years at one location. White-footed mice (*Peromyscus leucopus*) and Eastern chipmunks (*Tamias striatus*) were the most competent reservoirs for infection with the *A. phagocytophilum* human-active strain. Across the county, prevalence in ticks for both strains together was 8.3%; ticks were more than two times as likely to be infected with *A. phagocytophilum* human-active as *A. phagocytophilum* variant 1.

INTRODUCTION

Anaplasmosis is a rapidly emerging infectious disease in the United States, with over 1,700 cases reported in 2010—a 50% increase in the number of cases reported in the previous year and five times the number reported in 2000.[1] Most reported cases of anaplasmosis in the United States are concentrated in north central and northeastern states, although cases have been documented in 35 of 50 states.[2,3] Anaplasmosis has also been documented throughout Europe and Asia.[4–8]

Patients with anaplasmosis typically present with non-specific febrile symptoms, including fever, chills, headache, and myalgia,[9,10] particularly during summer months.[2,3] Most cases of anaplasmosis respond well to antibiotic treatment, but 17–56% of patients with anaplasmosis are hospitalized, and an estimated 1% of cases prove fatal.[10] Because of difficulties in diagnosis and lack of awareness of anaplasmosis by physicians and the public, many cases are misdiagnosed,[11] and national statistics almost certainly dramatically underreport this disease.[3,12]

Anaplasmosis is caused by a rickettsial bacterium *Anaplasma phagocytophilum*, groups of which form dense aggregates (morulae) in granulocytes.[13,14] The bacterium is passed from host to host through the bite of an infected ixodid tick—*Ixodes scapularis* in the eastern and central United States, *I. pacificus* in the western United States, and other ixodid ticks in Europe and Asia.[10,15,16] A number of vertebrate species has been shown through serology to be exposed to *A. phagocytophilum* in nature.[17–24] However, the relevance of serological data to transmission dynamics is limited, because seropositive individuals might not be currently infected and therefore, might not be infectious to feeding ticks. In the northeastern United States, actual rates of infection or transmission from infected wild hosts to naïve ticks have recently been evaluated for a suite of hosts from eastern deciduous forests.[25] This measure, called the realized reservoir competence, combines the probability that a particular host species will be infected and the probability that it will transmit the infection to feeding ticks.[25] Short-tailed shrews (*Blarina brevicauda*), white-footed mice (*Peromyscus leucopus*), and Eastern chipmunks (*Tamias striatus*) had the highest mean realized reservoir competence, infecting 10–15% of feeding ticks. Other hosts, including American robins (*Turdus migratorius*), raccoons (*Procyon lotor*), and opossums (*Didelphis virginiana*), were poor reservoirs by this metric, infecting < 5% of feeding ticks. All species tested were capable of transmitting *A. phagocytophilum* to feeding ticks, which is in contrast to earlier reports,[26] although earlier results were based on smaller sample sizes.

From an epidemiological point of view, not all strains of *A. phagocytophilum* are equally important. There are multiple strains of *A. phagocytophilum* in vertebrate hosts,[27–31] but only one strain seems to infect humans in the northeastern United States.[30] White-footed mice have been reported to be competent reservoirs for the human-infectious strain, called *A. phagocytophilum* human-active (*A. phagocytophilum*-ha), but not the other major strain, *A. phagocytophilum* variant 1 (*A. phagocytophilum*-v1). This conclusion was reached, because mice did not transmit *A. phagocytophilum*-v1 to feeding ticks, despite previous exposure to that strain in the laboratory.[29] In contrast, ticks feeding on white-tailed deer (*Odocoileus virginianus*) are infected with *A. phagocytophilum*-v1 more frequently than with *A. phagocytophilum*-ha.[32] This result may be because deer cannot support an infection with *A. phagocytophilum*-ha. In one study, almost 40% of ticks collected from deer were infected with *A. phagocytophilum*; of these ticks, 26% were carrying the *A. phagocytophilum*-ha strain.[32] However, blood samples collected from the same deer were never positive for *A. phagocytophilum*-ha.[32]

Despite the growing health concern about anaplasmosis in the United States and elsewhere, relatively little is known about interspecies variation in transmission of *A. phagocytophilum*-ha and *A. phagocytophilum*-v1, and little is known about the relative abundances of the two strains in questing ticks. We determined the realized reservoir competence for strains *A. phagocytophilum*-ha and *A. phagocytophilum*-v1 of 14 species of common vertebrate hosts for ticks. We then compared these realized reservoir competence data with the relative abundances of the two strains in host-seeking ticks collected

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from 146 different locations in a single county over 2 years in the Hudson Valley of New York, where anaplasmosis is endemic.

METHODS

Collecting ticks from hosts. To determine realized reservoir competence for the two strains, we trapped host individuals from 10 mammal species and 4 bird species using the methods described in detail elsewhere.[33] Briefly, hosts were captured on the property of the Cary Institute of Ecosystem Studies in Millbrook, New York during the peak abundance of larval black-legged ticks (*I. scapularis*) from July to September in 2008, 2009, and 2010. Captured individuals were held for 3 days in cages with wire mesh floors suspended over pans lined with wet paper towels so that ticks could feed to repletion and drop from hosts.

In some cases, if hosts did not drop > 10 ticks within 3 days, we infested them with unfed larval ticks. In these cases, each host was inoculated with larval ticks that had been either collected in the field or hatched from eggs in the laboratory. Larvae hatched from eggs were the offspring of adult ticks that had been collected from the area of the study and subsequently fed on rabbits. Transovarial transmission of *A. phagocytophilum* is not known to occur,[34] and therefore, larval ticks are uninfected; these infestations should not affect host exposure to the pathogen. During infestations, mice and birds were restrained by hand, whereas all other hosts were restrained in nylon mesh handling cones. Infestations were conducted by placing ticks on the host's neck and head with a #00 paintbrush. Hosts that had been infested were held for an additional 4 days, and engorged ticks were collected each day.

Engorged larvae were held in moistened glass vials until they molted into the nymphal stage. Newly molted nymphs were flash-frozen in liquid nitrogen and stored at –80°C. All animal care and husbandry were conducted with approval from the Cary Institute of Ecosystem Studies Institutional Animal Care and Use Committee.

In general, we only assessed realized reservoir competence of hosts that produced a minimum of 10 newly molted nymphs. However, three species—*Glaucomys volans*, *Sorex cinereus*, and *Mephitis mephitis*—had low body burdens. For these species, we tested ticks from individuals with greater than four newly molted nymphs; we consider these data provisional because of the low number of ticks per individual host.

Collecting ticks from forests. Between 2007 and 2012, we collected questing nymphal ticks from three long-term plots at the Cary Institute of Ecosystem Studies in Millbrook, New York. Forests at the Cary Institute are typical of the eastern deciduous forests of New York and New England. Plots are dominated by oaks (*Quercus rubra* and *Q. prinus*) in the overstory, with primarily oak and sugar maple (*Acer saccharum*) seedlings, maple-leaved viburnum (*Viburnum acerifolium*), witch hazel (*Hamamelis virginiana*), and ironwood (*Ostrya virginiana*) in the understory. One 2.25-ha plot (150 × 150 m) was established in 1991, and two more plots were added in 1995 to comprise three plots, with more than 700 m separating pairs.

We also sampled questing nymphal ticks in June of 2011 (148 sites) and June of 2012 (78 sites) in forested locations throughout Dutchess County, New York. For county locations, we selected sites using a Geographic Information Systems (GIS) map of forested and non-forested land cover digitized from aerial orthophotos generated in 2009. We generated an initial candidate list of 2,500 random points using a random point overlay. These points were then stratified by the percentage of forest cover in the surrounding landscape to provide equal representation along a gradient of forest cover from extensively forested to highly fragmented. We eliminated sites when access was poor or property owners could not be located or recruited.

At all sites in all years, we collected questing nymphal ticks by drag sampling.[35] Corduroy cloths (1 m^2) were dragged along 400-m transects in each site one or two times in a given year during the annual peak in nymphal questing activity. Ticks were collected from the cloths every 15–30 m. Questing nymphs were flash-frozen on collection as described above. All sites sampled for questing nymphs were in eastern deciduous forests. To estimate prevalence at each site, we tested 10–30 ticks.

Extracting and amplifying DNA. Total genomic DNA was extracted from ticks using either the DNeasy or DNeasy 96 Blood and Tissue Kit (Qiagen, Hilden, Germany) or the Gentra PureGene Tissue Kit (Qiagen, Hilden, Germany). To amplify extracted DNA, we followed established protocols.[25,36] Briefly, we used primers ApMSP2f and ApMSP2r and probe ApMSP2p, which are specific to the *msp2* gene of *A. phagocytophilum* and generate a 77-base pair (bp) fragment. Real-time polymerase chain reaction (PCR) was performed in a Bio-Rad CFX96 Real-Time PCR System (Bio-Rad, Hercules, CA). We used DNA extractions from unfed larval ticks and ultrapure water as negative controls. The cloned 77-bp target was used as a positive control. Barrier pipette tips were used throughout the process to prevent contamination.

For each tick, we conducted three replicate assays using PCR. As described fully elsewhere,[25] ticks were considered positive for *A. phagocytophilum* if any one of three replicate samples was called positive by default settings, meaning that it amplified relative to negative controls. We conducted additional confirmatory tests for any ticks with marginal results (i.e., moderate fluorescence). If any replicate was positive in the confirmatory tests, ticks were considered positive for *A. phagocytophilum*; if all three replicates in the confirmatory test were marginal or negative, ticks were considered negative.

Determining strain identity. Ticks that were positive for *A. phagocytophilum* in these initial tests were subsequently tested for the identity of the strain that they carried using nested PCR. In the primary round, we used primers ge3a and ge2[37] to amplify a 546-bp segment of the 16S gene. The product from this round was used as a template in a second round of PCR, in which primers ge9f[37] and ge9r (5′-TTA CTC ACC CGT CTG CCA CT-3′; designed for this study) were used to amplify a 58-bp product. Primary-round PCRs were performed in 20-μL volumes with final concentrations of 1× Promega PCR Master Mix (Promega, Madison, WI) and 0.5 μM primers. Thermal cycling was performed with an initial denaturing period of 95°C for 2 minutes, then 40 cycles of 94°C for 30 seconds, 55°C for 1 minute, and 72°C for 1 minute, and a final extension of 72°C for 5 minutes. Secondary-round PCRs were performed in 20-μL volumes with final concentrations of 1× iQ SYBR Green Supermix (Bio-Rad, Hercules, CA) and 0.5 μM primers. Thermal cycling was performed with an initial denaturing period of 95°C for 10 minutes, then 40 cycles of 95°C for 15 seconds and 60°C for 1 minute followed by a melt curve analysis, in which the temperature increased from 76°C to 85°C at 0.2°C every 10 seconds.

TABLE 1

Sample size, realized reservoir competence, and transmission probabilities for 14 common hosts for two strains of *A. phagocytophilum*: *A. phagocytophilum*-ha (*Ap*-ha) and *A. phagocytophilum*-v1 (*Ap*-v1)

Species	*N* hosts	*N* ticks	Mean (SE) realized reservoir competence		Mean percentage of individual hosts transmitting		
			Ap-ha	*Ap*-v1	*Ap*-ha	*Ap*-v1	Both
Eastern chipmunk *T. striatus*	23	369	6.78% (2.00%)	0.58% (0.31%)	26.1%	8.7%	8.7%
White-footed mouse *P. leucopus*	38	748	6.65% (1.98%)	0.94% (0.61%)	15.8%	2.6%	2.6%
Red squirrel *Tamiasciurus hudsonicus*	15	297	4.92% (4.22%)	0.00% (0.00%)	20.0%	0.00%	0.00%
Short-tailed shrew *B. brevicauda*	29	546	3.64% (1.51%)	2.22% (1.05%)	20.7%	13.8%	10.3%
Catbird *Dumetella carolinensis*	18	299	3.33% (1.92%)	0.00% (0.00%)	5.6%	0.0%	0.0%
Flying squirrel* *G. volans*	7	87	2.00% (1.85%)	2.38% (2.20%)	14.3%	14.3%	0.0%
Gray squirrel *Sciurus carolinensis*	20	358	1.74% (0.79%)	1.02% (0.55%)	20.0%	15.0%	5.0%
Masked shrew* *S. cinereus*	6	41	1.67% (1.67%)	1.67% (1.67%)	16.7%	16.7%	16.7%
Raccoon *P. lotor*	26	503	1.40% (0.60%)	0.38% (0.24%)	15.4%	7.7%	3.9%
American robin *T. migratorius*	20	345	1.40% (0.76%)	0.42% (0.32%)	10.0%	5.0%	0.0%
Veery *Catharus fuscescens*	22	445	1.17% (0.55%)	0.00% (0.00%)	13.6%	0.0%	0.0%
Wood thrush *Hylocichla mustelina*	28	496	0.89% (0.59%)	0.00% (0.00%)	7.1%	0.0%	0.0%
Opossum *D. virginiana*	27	533	0.53% (0.37%)	1.05% (0.68%)	7.4%	11.1%	3.7%
Striped skunk* *Mephitis mephitis*	2	31	0.00% (0.00%)	4.76% (0.00%)	0.0%	50.0%	0.0%

Realized reservoir competence is measured as the mean percentage of uninfected ticks that became infected from feeding on an individual wild host.
*Small sample size.

The melt temperature for *A. phagocytophilum*-ha was 78.6–79.8°C, whereas for *A. phagocytophilum*-v1, it was 81.6–83.4°C. We did not observe consistent melt temperatures other than those melt temperatures in the ranges indicated above. To verify these melt temperatures, we sequenced the DNA from 10 questing nymphs from each strain category. Each DNA segment was cloned before sequencing; the number of clones per DNA ranged from 25 to 40 (*A. phagocytophilum*-ha: mean = 32.6; *A. phagocytophilum*-v1: mean = 37.6). For samples identified as *A. phagocytophilum*-v1, 98% of clones were identical to *A. phagocytophilum*-v1 (accession no. AY193887 in GenBank) (Supplemental Table 1). The remaining 2% of clones differed from the *A. phagocytophilum*-v1 sequence by 1 bp (Supplemental Table 1), and none of the clones matched the sequence identified as *A. phagocytophilum*-ha. For *A. phagocytophilum*-ha, 70% of 317 total clones were identical to *A. phagocytophilum*-ha (accession no. U02521 in GenBank) (Supplemental Table 1). The remaining clones differed from *A. phagocytophilum*-ha in GenBank by 1 or 2 bp, and none of the clones matched the sequence identified as *A. phagocytophilum*-v1. The identification of clones that differed from the *A. phagocytophilum*-ha reference sequence but are not *A. phagocytophilum*-v1 suggests the possibility of a complex of strains of *A. phagocytophilum*-ha. Our method did not allow detection of coinfection of both strains in one tick.

Estimating infection prevalence. Realized reservoir competence for each host species was calculated as the mean percentage of ticks infected per individual host. This measure incorporates natural variation among species in infection with *A. phagocytophilum* as well as variation among species in the probability of transmitting infection to feeding ticks.[38] We used likelihood-based methods to separately estimate prevalence of infection of both strains among hosts as well as each host's propensity to transmit the infection to ticks given an infection.[38] At our long-term plots at the Cary Institute, we used the pool of ticks collected on the three plots to calculate the mean site-wide infection prevalence of questing nymphal ticks for each year. At our county sites, we calculated the mean infection prevalence of questing nymphs from each site, including only those sites where we were able to collect ≥ 10 nymphs in either year. To determine if the infection prevalence of questing nymphal ticks at county sites was correlated between years, we calculated the correlation coefficient of tick infection prevalence for each strain. For this analysis, we excluded sites at which no evidence of either *A. phagocytophilum* strain was detected in either year.

RESULTS

To determine realized reservoir competence of host species for the two strains of *A. phagocytophilum*, we tested a total of 5,098 nymphal ticks from 281 individuals of 14 host species. Overall, 2.7% of ticks were infected with strain *A. phagocytophilum*-ha, and 0.8% of ticks were infected with strain *A. phagocytophilum*-v1. As previously reported, host species varied in the probability that they would transmit *A. phagocytophilum* to feeding ticks (Figure 1).[25] Hosts also varied in the proportion of ticks that they infected with the two strains (Figure 1). White-footed mice and Eastern chipmunks, for example, were relatively likely to transmit *A. phagocytophilum*-ha, with mice infecting 6.7% (±0.6% SEM) and chipmunks infecting 6.8% (±2.0%) of feeding larval ticks. However, these species were relatively unlikely to transmit *A. phagocytophilum*-v1, with both species infecting < 1% of feeding larvae (Table 1). In contrast, all ticks that acquired infection from striped skunks and the majority of ticks that acquired infection from Virginia opossums and southern flying squirrels were infected with *A. phagocytophilum*-v1. Of 281 hosts from which we tested ticks, 10 individuals transmitted both *A. phagocytophilum*-ha and *A. phagocytophilum*-v1 to feeding ticks. These 10 individuals represented 7 of 14 species of hosts that we identified (Table 1). In particular, the two species of shrews were more likely than other taxa to transmit both strains (10% of *Blarina* and 17% of *Sorex*) (Table 1). In addition, 9% of chipmunks transmitted both strains. The remaining host species, including mice and three species of ground-nesting songbirds, either never or rarely transmitted both strains (Table 1). For 2% of infected ticks, we detected infection with *A. phagocytophilum* but could not determine strain identity using our assay.

We differentiated realized reservoir competence into its two components, prevalence and infectivity, as described in Methods. Estimates of prevalence for each species varied from 15% to 100% for *A. phagocytophilum*-ha, with a great deal of

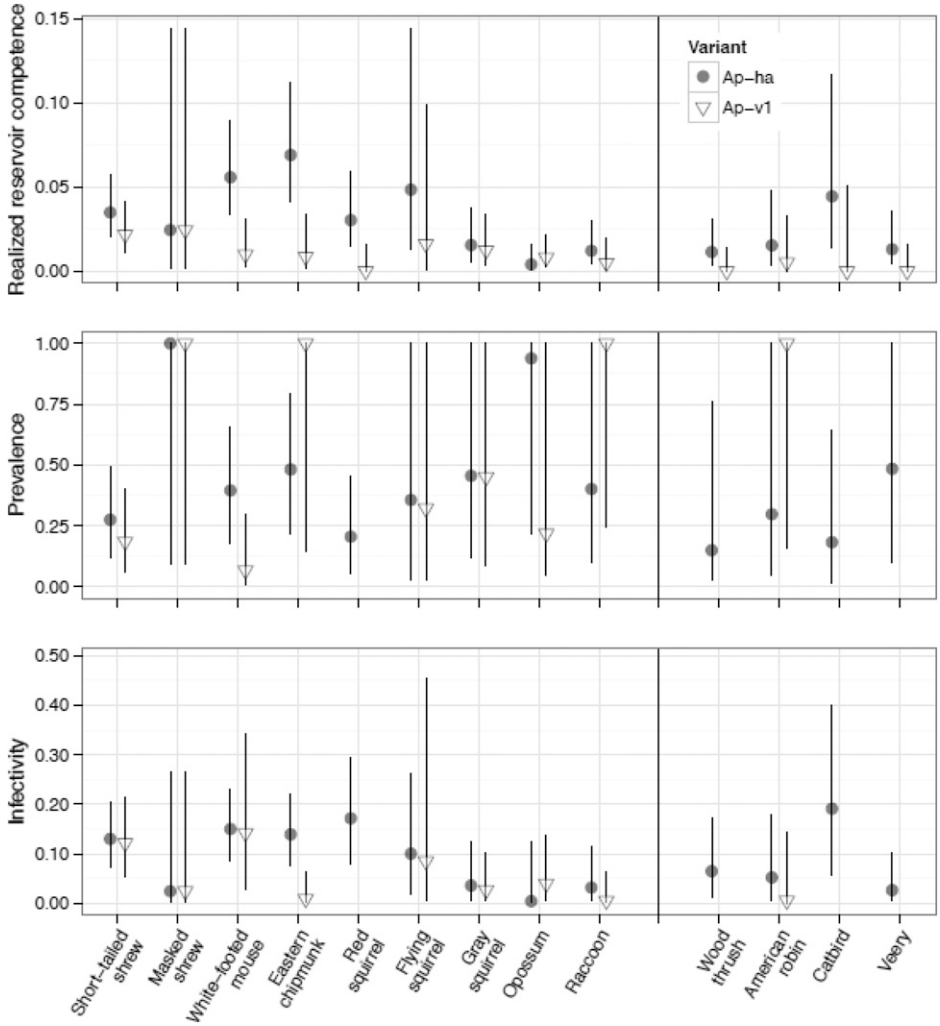

FIGURE 1. Realized reservoir competence, prevalence of infection, and infectivity to ticks of 14 species of vertebrate hosts for two strains of *A. phagocytophilum*, *A. phagocytophilum*-ha (gray) and *A. phagocytophilum*-v1 (white), showing means and 95% confidence intervals (lines). Realized reservoir competence is the mean percentage of uninfected larval ticks that became infected after feeding on naturally infected individuals. Prevalence is the probability that an individual of the host species is infected; infectivity is the probability that an infected host will pass infection to a feeding tick. *A. phagocytophilum*-ha is known to cause infections in humans, whereas *A. phagocytophilum*-v1 is not.

uncertainty, and from 0% to 100% for *A. phagocytophilum*-v1 (Figure 1). Estimates of infectivity were generally low and well-constrained (Figure 1). Infectivity varied considerably among species, with short-tailed shrews, mice, chipmunks, red squirrels, and catbirds that were infected with *A. phagocytophilum*-ha transmitting the infection to roughly 10–15% of the ticks that fed on them (Figure 1). The other birds—American robins, veeries, and wood thrushes—as well as opossums, raccoons, gray squirrels, and *Sorex* shrews were much less likely to pass on the infection if they were infected. There was a tendency for lower infectivity of *A. phagocytophilum*-v1 compared with *A. phagocytophilum*-ha, although this result was within the range of the confidence intervals for all species except chipmunks.

In 2011, we collected > 10 ticks at 134 of 148 county-wide sites, giving us a sufficient sample size to estimate *A. phagocytophilum* infection prevalence at these locations. In 2012, overall tick abundance was lower, and we were able to collect sufficient sample sizes at only 60 sites, 46 of which were sites that we had also sampled in 2011. Averaging results across both years, 8.3% (±0.6% SEM) of 4,152 ticks were positive for *A. phagocytophilum*, with 4.0% (±0.4%) infected with *A. phagocytophilum*-ha and 2.4% (±0.3%) infected with *A. phagocytophilum*-v1. The remaining 1.9% of ticks tested positive for *A. phagocytophilum*, but the strain could not be determined from our assay. For both years combined, at least one tick was positive for *A. phagocytophilum*-ha at 41% of sites, and at least one tick was positive for *A. phagocytophilum*-v1 at 30% of sites (Figure 2); 60 of 194 total sites (31%) had no *A. phagocytophilum* of either strain. The prevalence of ticks infected across 2 years was significantly correlated for *A. phagocytophilum*-ha ($F_{1,44}$ = 22.2; $P << 0.001$) but not for *A. phagocytophilum*-v1 ($F_{1,44}$ = 22.2; $P = 0.11$) (Figure 3).

From our long-term grids at the Cary Institute, we tested 890 questing nymphal ticks over 6 years. Of these ticks, an overall mean of 4.9% were infected with *A. phagocytophilum*-ha, and only 0.6% were infected with *A. phagocytophilum*-v1.

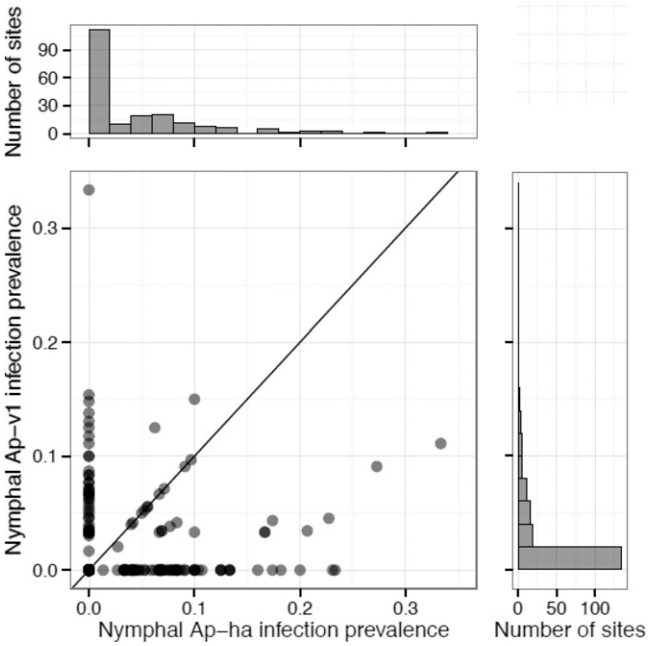

FIGURE 2. Correlations of the prevalence of infection in questing nymphal ticks of two strains of *A. phagocytophilum* at 148 sites in Dutchess County sampled in 2011 and 2012. Strain *A. phagocytophilum*-ha (*Ap*-ha) is known to cause infection in humans, whereas strain *A. phagocytophilum*-v1 (*Ap*-v1) is not. Histograms represent the prevalence of *A. phagocytophilum*-ha (horizontal histogram) and *A. phagocytophilum*-v1 (vertical histogram) for 2011 and 2012 data combined.

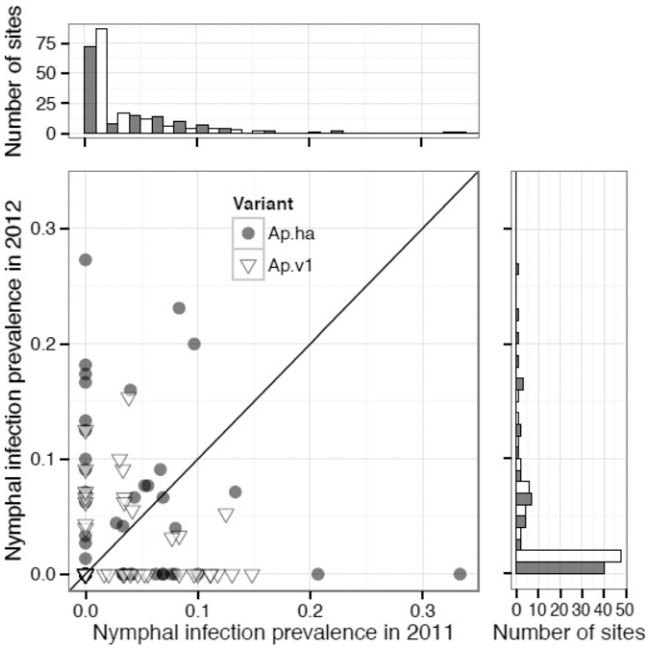

FIGURE 3. Correlations between years of the prevalence of infection in questing nymphal ticks of *A. phagocytophilum*-ha (*Ap*.ha; gray circles) and *A. phagocytophilum*-v1 (*Ap*.v1; white triangles) at 148 sites in Dutchess County. Histograms represent the prevalence of *A. phagocytophilum*-ha (gray bars) and *A. phagocytophilum*-v1 (white bars) for 2011 (horizontal histogram) and 2012 (vertical histogram).

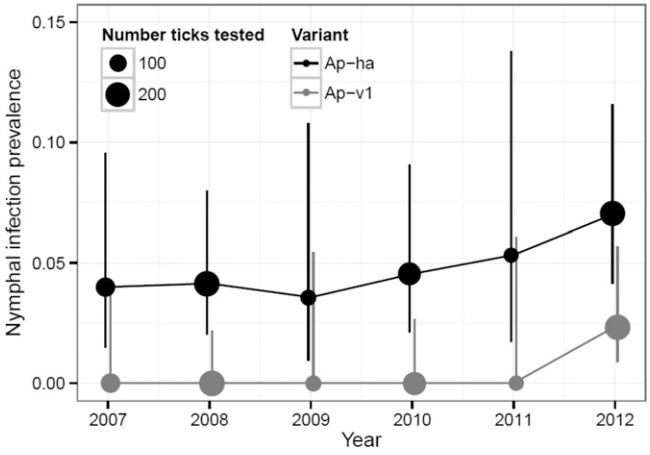

FIGURE 4. Mean percentages, with 95% confidence intervals, of questing nymphal ticks infected with each of two strains of *A. phagocytophilum* over 6 years at a study site in Dutchess County, New York. *A. phagocytophilum*-ha (*Ap*-ha; black circles) is known to cause infection in humans, whereas *A. phagocytophilum*-v1 (*Ap*-v1; gray circles) is not. The number of ticks sampled is indicated by the size of the symbol.

No ticks tested positive for *A. phagocytophilum*-v1 until 2012 (Figure 4).

DISCUSSION

Across a heterogeneous landscape in Dutchess County, New York, 8.3% of ticks were infected with *A. phagocytophilum*. These *A. phagocytophilum*-positive ticks were more than two times as likely to be infected with the human-active strain complex, *A. phagocytophilum*-ha, as with *A. phagocytophilum*-v1, which does not seem to cause infections in humans. Over 6 years of monitoring at a single location, we found no apparent trend in the prevalence of *A. phagocytophilum* for either strain individually or both strains combined (Figure 4). Ten of fourteen host species that we tested, including white-footed mice, Eastern chipmunks, two species of shrews, and gray squirrels, were capable of transmitting both strains to feeding ticks. Mice and chipmunks had the highest realized reservoir competence for *A. phagocytophilum*-ha, whereas striped skunks were the most competent realized reservoirs for *A. phagocytophilum*-v1, although the latter observation is based on a sample size of two. Skunks were the only species in our study that transmitted only *A. phagocytophilum*-v1 to ticks. Separating realized reservoir competence into its two components, prevalence and infectivity, it is not clear whether the prevalence of *A. phagocytophilum* infection varied among host species, but it is clear that, given an infection, some species (e.g., white-footed mice and red squirrels) were more likely to pass on the infection than others (e.g., opossums and raccoons) (Figure 1).

A prior laboratory investigation suggested that *A. phagocytophilum*-v1 could not establish an infection in white-footed mice.[29] We found that 1% of uninfected ticks feeding on mice acquired *A. phagocytophilum*-v1, although the majority of ticks that were infected by mice acquired *A. phagocytophilum*-ha (Table 1). The difference in these results may be because of relatively small sample sizes in the laboratory study combined with low reservoir competence of

mice for *A. phagocytophilum*-v1. In their experimental feeding study, only one to two ticks that became infected with *A. phagocytophilum*-v1 had fed to repletion on the mice.[30] Our mice were caught in the field, and infections had occurred naturally by ticks feeding at natural densities, which may have given them higher rates of exposure than in the laboratory study. Based on our results, mice can acquire and transmit *A. phagocytophilum*-v1, although they are more likely to transmit *A. phagocytophilum*-ha.

In previous research on *A. phagocytophilum*, mice that had been infected with *A. phagocytophilum* in the laboratory were less susceptible to *A. phagocytophilum* on subsequent exposure.[39] Previous work had also suggested that infection with *A. phagocytophilum* might be transient in rodents, because laboratory mice (*Mus musculus*) could clear infection.[40] Together, these results suggested that white-footed mice might not play a large role in infecting ticks in nature.[39,41] Our results show that white-footed mice have among the highest realized reservoir competence for larval ticks feeding during the natural cycle of tick infection. Both the ability of mice to maintain and transmit *A. phagocytophilum* infection and their relatively high levels of realized reservoir competence improve our understanding of the contribution of mice to *A. phagocytophilum* prevalence in ticks in natural settings. Because of their relatively high abundance[35] and high permissiveness to tick feeding,[34] mice seem likely to contribute the majority of nymphs infected with *A. phagocytophilum*. This hypothesis is supported by our observation that white-footed mice and Eastern chipmunks were the only hosts that transmitted infection to feeding larval ticks at rates (~7%) (Figure 1) that could produce the values of nymphal infection prevalence that we observed for questing ticks (~8%).

Because of logistical constraints, we did not assess the realized reservoir competence of white-tailed deer for the two strains of *A. phagocytophilum*. Individual deer can host > 200 feeding larval ticks at one time.[42] In a previous study, blood from white-tailed deer was tested for the presence of the two strains[32]; 11 of 38 deer were positive for *A. phagocytophilum*-v1, whereas no *A. phagocytophilum*-ha was found in deer blood, suggesting that they can acquire *A. phagocytophilum*-v1 but perhaps not *A. phagocytophilum*-ha.[32] In the same study, feeding adult ticks collected from deer were found to be infected with *A. phagocytophilum*-v1 more often than with *A. phagocytophilum*-ha.[32] However, it is unknown whether these infections were acquired from deer or another species during a previous blood meal.

For our landscape-level investigation, we found that ~8% of ticks from the landscape-level study were infected with *A. phagocytophilum*, and two-thirds of these were infected with the human-active strain. This result incorporates the effects of deer in the relative abundance of the two strains. Because a majority of these ticks are infected with the human-active strain rather than the variant 1 strain, hosts other than deer must be responsible for providing many larval tick meals at our sites.

The relative abundance of the two strains of *A. phagocytophilum* in questing ticks from sites in the eastern United States varies substantially. In Rhode Island, *A. phagocytophilum*-v1 was relatively common, comprising up to 80% of questing nymphal and adult ticks that were positive for *A. phagocytophilum*, whereas in parts of neighboring Connecticut, questing nymphal and adult ticks were only infected with *A. phagocytophilum*-ha.[28] In northwestern Pennsylvania, all *A. phagocytophilum*-positive ticks harbored *A. phagocytophilum*-ha, whereas in southeastern Pennsylvania, most ticks infected with *A. phagocytophilum* had strain *A. phagocytophilum*-v1.[43] A survey of a suite of tick-borne pathogens in four states (Pennsylvania, Maine, Wisconsin, and Indiana) found *A. phagocytophilum* in a total of 36 adult ticks.[44] Of the total pool of *A. phagocytophilum*-infected ticks from four states, 35 ticks were infected with *A. phagocytophilum*-ha, and only 1 tick was infected with *A. phagocytophilum*-v1.[44] Here, we show that the range of variation within a single county spans the range of variation previously seen between states in the northeastern and midwestern United States. In the one local site that we monitored for 6 consecutive years, *A. phagocytophilum*-v1 was absent from our samples until the final year of the study (Figure 4), suggesting that individual sites might not be accurately characterized by short-term sampling.

Because our data on realized reservoir competence show that some hosts are more likely to transmit one of the strains than the other strain, one possible explanation for wide variation in the relative abundance of strains at a county scale is that these sites vary in host community composition. For example, host communities dominated by white-footed mice and chipmunks should produce a large proportion of ticks infected with *A. phagocytophilum*-ha (Figure 1). In contrast, ticks feeding in a host community composed of flying squirrels, opossums, and skunks should tend to be infected with the *A. phagocytophilum*-v1 strain. Whether the variation that we observed in strain prevalence can be explained by variation in the host community cannot be determined without investigating how strain frequencies vary with known host community composition. However, in previous research investigating vertebrate community composition at multiple sites across three eastern states, we have found mice at all sites, chipmunks at nearly all the sites, and other species present only in forests with higher diversity.[45] Thus, one possible explanation for a high relative abundance of *A. phagocytophilum*-ha is the presence of a low-diversity host community dominated by mice and chipmunks. This area is an important area for additional research.

In our study, nymphal infection with the two strains at individual sites varied considerably between years. Such variation could be caused by changes in the composition of the host community over time. The abundance of small mammals, which are important reservoirs for *A. phagocytophilum*, is known to fluctuate from year to year based on the availability of resources, particularly acorn mast.[46] If the host community varies significantly from year to year, we would expect the infection prevalence of ticks with *A. phagocytophilum* strains to vary from year to year as well. An alternative explanation for the variation in *A. phagocytophilum* prevalence through time is that stochastic factors lead to pronounced fluctuations in prevalence. The relatively low prevalence of *A. phagocytophilum* at our sites is consistent with stochasticity, which has a bigger effect when prevalence levels are low.

Substantial variation exists in *A. phagocytophilum* strains found in humans,[47] wildlife,[24,48,49] and questing ticks.[50,51] We have focused on two of these strains, one of which is found in many wildlife hosts and humans (*A. phagocytophilum*-ha) and the other one of which is found in many wildlife hosts but not humans (*A. phagocytophilum*-v1). Future research should

continue to investigate the origins and consequences of strain diversity in *A. phagocytophilum*, particularly because it affects variation in human risk of exposure to this emerging pathogen.

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REFERENCES

1. Centers for Disease Control, 2013. *Statistics and Epidemiology*. Available at: http://www.cdc.gov/anaplasmosis/stats/. Accessed August 20, 2013.
2. McQuiston JH, Paddock CD, Holman RC, Childs JE, 1999. The human ehrlichioses in the United States. *Emerg Infect Dis 5:* 635–642.
3. Demma LJ, Holman RC, McQuiston JH, Krebs JW, Swerdlow DL, 2005. Epidemiology of human ehrlichiosis and anaplasmosis in the United States, 2001–2002. *Am J Trop Med Hyg 73:* 400–409.
4. Ogden NH, Brown K, Horrocks BK, Woldehiwet Z, Bennett M, 1998. Granulocytic ehrlichia infection in ixodid ticks and mammals in woodlands and uplands of the U.K. *Med Vet Entomol 12:* 423–429.
5. Petrovec M, Bidovec A, Sumner JW, Nicholson WL, Childs JE, Avšič-Županc T, 2002. Infection with *Anaplasma phagocytophila* in cervids from Slovenia: evidence of two genotype lineages. *Wien Klin Wochenschr Middle Eur J Med 114:* 641–647.
6. Cao W, Zhao Q, Zhang P, Yang H, Wu X, Wen B, Zhang X, Habbema JDF, 2003. Prevalance of *Anaplasma phagocytophila* and *Borrelia burgdorferi* in *Ixodes persulcatus* ticks from northeastern China. *Am J Trop Med Hyg 68:* 547–550.
7. Cao W, Zhan L, He J, Foley JE, De Vlas SJ, Wu X, Yang H, Richardus JH, Habbema JDF, 2006. Natural *Anaplasma phagocytophilum* infection of ticks and rodents from a forest area of Jilin Province, China. *Am J Trop Med Hyg 75:* 664–668.
8. Kim CM, Yi YH, Yu DH, Lee MJ, Cho MR, Desai AR, Shringi S, Klein TA, Kim HC, Song JW, Baek LJ, Chong ST, O'guinn ML, Lee JS, Lee IY, Park JH, Foley J, Chae JS, 2006. Tick-borne rickettsial pathogens in ticks and small mammals in Korea. *Appl Environ Microbiol 72:* 5766–5776.
9. Ramsey AH, Belongia EA, Gale CM, Davis JP, 2002. Outcomes of treated human granulocytic ehrlichiosis cases. *Emerg Infect Dis 8:* 398–401.
10. Jin H, Wei F, Liu Q, Qian J, 2012. Epidemiology and control of human granulocytic anaplasmosis: a systematic review. *Vector Borne Zoonotic Dis 12:* 269–274.
11. Chowdri HR, Gugliotta JL, Berardi VP, Goethert HK, Molloy PJ, Sterling SL, Telford SR, 2013. *Borrelia miyamotoi infection presenting as human granulocytic anaplasmosis: a case report. Ann Intern Med 159:* 21–27.
12. Dahlgren FS, Mandel EJ, Krebs JW, Massung RF, McQuiston JH, 2011. Increasing incidence of *Ehrlichia chaffeensis* and *Anaplasma phagocytophilum* in the United States, 2000–2007. *Am J Trop Med Hyg 85:* 124.
13. Bakken JS, Krueth J, Wilson-Nordskog C, Tilden RL, Asanovich K, Dumler JS, 1996. Clinical and laboratory characteristics of human granulocytic ehrlichiosis. *JAMA 275:* 199–205.
14. Dumler JS, Choi KS, Garcia-Garcia JC, Barat NS, Scorpio DG, Garyu JW, Grab DJ, Bakken JS, 2005. Human granulocytic anaplasmosis and *Anaplasma phagocytophilum*. *Emerg Infect Dis 11:* 1828–1834.
15. Hodzic E, Fish D, Maretzki CM, De Silva AM, Feng S, Barthold SW, 1998. Acquisition and transmission of the agent of human granulocytic ehrlichiosis by *Ixodes scapularis* ticks. *J Clin Microbiol 36:* 3574–3578.
16. Kramer VL, Randolph MP, Hui LT, Irwin WE, Gutierrez AG, Vugia DJ, 1999. Detection of the agents of human ehrlichioses in ixodid ticks from California. *Am J Trop Med Hyg 60:* 62–65.
17. Walls JJ, Greig B, Neitzel DF, Dumler JS, 1997. Natural infection of small mammal species in Minnesota with the agent of human granulocytic ehrlichiosis. *J Clin Microbiol 35:* 853–855.
18. Nicholson WL, Muir S, Sumner JW, Childs JE, 1998. Serologic evidence of infection with *Ehrlichia* spp. in wild rodents (Muridae: Sigmodontinae) in the United States. *J Clin Microbiol 36:* 695–700.
19. Nicholson WL, Castro MB, Kramer VI, Sumner JW, Childs JE, 1999. Dusky-footed wood rats (*Neotoma fuscipes*) as reservoirs of granulocytic ehrlichiae (Rickettsiales: Ehrlichieae) in northern California. *J Clin Microbiol 37:* 3323–3327.
20. Magnarelli LA, Ijdo JW, Stafford KC III, Fikrig E, 1999. Infections of granulocytic ehrlichiae and *Borrelia burgdorferi* in white-tailed deer in Connecticut. *J Wildl Dis 35:* 266–274.
21. Levin ML, Nicholson WL, Massung RF, Sumner JW, Fish D, 2002. Comparison of the reservoir competence of medium-sized mammals and *Peromyscus leucopus* for *Anaplasma phagocytophilum* in Connecticut. *Vector Borne Zoonotic Dis 2:* 125–136.
22. Goethert HK, Telford SR III, 2003. Enzootic transmission of the agent of human granulocytic ehrlichiosis among cottontail rabbits. *Am J Trop Med Hyg 68:* 633–637.
23. Dugan VG, Yabsley MJ, Tate CM, Mead DG, Munderloh UG, Herron MJ, Stallknecht DE, Little SE, Davidson WR, 2006. Evaluation of white-tailed deer (*Odocoileus virginianus*) as natural sentinels for *Anaplasma phagocytophilum*. *Vector Borne Zoonotic Dis 6:* 192–207.
24. Foley J, Nieto NC, Madigan J, Sykes J, 2008. Possible differential host tropism in *Anaplasma phagocytophilum* strains in the western United States. *Ann N Y Acad Sci 1149:* 94–97.
25. Keesing F, Hersh MH, Tibbetts M, McHenry DJ, Duerr S, Brunner J, Killilea M, LoGiudice K, Schmidt KA, Ostfeld RS, 2012. Reservoir competence of vertebrate hosts for *Anaplasma phagocytophilum*. *Emerg Infect Dis 18:* 2013–2016.
26. Levin ML, Fish D, 2000. Acquisition of coinfection and simultaneous transmission of *Borrelia burgdorferi* and *Ehrlichia phagocytophila* by *Ixodes scapularis* ticks. *Infect Immun 68:* 2183–2186.
27. Belongia EA, Reed KD, Mitchell PD, Kolbert CP, Persing DH, Gill JS, Kazmierczak JK, 1997. Prevalence of granulocytic ehrlichia infection among white-tailed deer in Wisconsin. *J Clin Microbiol 35:* 1465–1468.
28. de la Fuente J, Massung RF, Wong SJ, Chu FK, Lutz H, Meli M, Loewenich FD, Grzeszczuk A, Torina A, Caracappa S, Mangold AJ, Naranjo V, Stuen S, Kocan KM, 2005. Sequence analysis of the msp4 gene of *Anaplasma phagocytophilum* strains. *J Clin Microbiol 43:* 1309–1317.
29. Massung RF, Priestley RA, Miller NJ, Mather TN, Levin ML, 2003. Inability of a variant strain of *Anaplasma phagocytophilum* to infect mice. *J Infect Dis 188:* 1757–1763.
30. Massung RF, Mather TN, Priestley RA, Levin ML, 2003. Transmission efficiency of the AP-variant 1 strain of *Anaplasma phagocytophila*. *Ann N Y Acad Sci 990:* 75–79.
31. Rejmanek D, Bradburd G, Foley J, 2012. Molecular characterization reveals distinct genospecies of *Anaplasma phagocytophilum*

from diverse North American hosts. *J Med Microbiol 61:* 204–212.
32. Massung RF, Courtney JW, Hiratzka SL, Pitzer VE, Smith G, Dryden RL, 2005. *Anaplasma phagocytophilum* in white-tailed deer. *Emerg Infect Dis 11:* 1604–1606.
33. Hersh MH, Tibbetts M, Strauss M, Ostfeld RS, Keesing F, 2012. Reservoir competence of wildlife host species for *Babesia microti*. *Emerg Infect Dis 18:* 1951–1957.
34. Keesing F, Brunner J, Duerr S, Killilea M, LoGiudice K, Schmidt K, Vuong H, Ostfeld RS, 2009. Hosts as ecological traps for the vector of Lyme disease. *Proc Biol Sci 276:* 3911–3919.
35. Ostfeld RS, Canham CD, Oggenfuss K, Winchcombe RJ, Keesing F, 2006. Climate, deer, rodents, and acorns as determinants of variation in Lyme-disease risk. *PLoS Biol 4:* e145.
36. Courtney JW, Kostelnik LM, Zeidner NS, Massung RF, 2004. Multiplex real-time PCR for detection of *Anaplasma phagocytophilum* and *Borrelia burgdorferi*. *J Clin Microbiol 42:* 3164–3168.
37. Massung RF, Slater K, Owens JH, Nicholson WL, Mather TN, Solberg VB, Olson JG, 1998. Nested PCR assay for detection of granulocytic ehrlichiae. *J Clin Microbiol 36:* 1090–1095.
38. Brunner JL, LoGiudice K, Ostfeld RS, 2008. Estimating reservoir competence of *Borrelia burgdorferi* hosts: prevalence and infectivity, sensitivity, and specificity. *J Med Entomol 45:* 139–147.
39. Levin ML, Fish D, 2000. Immunity reduces reservoir host competence of *Peromyscus leucopus* for *Ehrlichia phagocytophila*. *Infect Immun 68:* 1514–1518.
40. Sun W, Ijdo JW, Telford SR, Hodzic E, Zhang Y, Barthold SW, Fikrig E, Telford SR III, 1997. Immunization against the agent of Human Granulocytic Ehrlichiosis in a murine model. *J Clin Invest 100:* 3014–3018.
41. Hodzic E, Fish D, Maretzki CM, De Silva AM, Feng S, Barthold SW, 1998. Acquisition and transmission of the agent of human granulocytic ehrlichiosis by *Ixodes scapularis* ticks. *J Clin Microbiol 36:* 3574–3578.
42. LoGiudice K, Ostfeld RS, Schmidt KA, Keesing F, 2003. The ecology of infectious disease: effects of host diversity and community composition on Lyme disease risk. *Proc Natl Acad Sci USA 100:* 567–571.
43. Courtney JW, Dryden RL, Montgomery J, Schneider BS, Smith G, Massung RF, 2003. Molecular characterization of *Anaplasma phagocytophilum* and *Borrelia burgdorferi* in *Ixodes scapularis* ticks from Pennsylvania. *J Clin Microbiol 41:* 1569–1573.
44. Steiner FE, Pinger RR, Vann CN, Grindle N, Civitello D, Clay K, Fuqua C, 2008. Infection and co-infection rates of *Anaplasma phagocytophilum* variants, *Babesia* spp., *Borrelia burgdorferi*, and the rickettsial endosymbiont in *Ixodes scapularis* (Acari: Ixodidae) from sites in Indiana, Maine, Pennsylvania, and Wisconsin. *J Med Entomol 45:* 289–297.
45. LoGiudice K, Duerr ST, Newhouse MJ, Schmidt KA, Killilea ME, Ostfeld RS, 2008. Impact of host community composition on Lyme disease risk. *Ecology 89:* 2841–2849.
46. Ostfeld RS, 2011. *Lyme Disease: The Ecology of a Complex System*. Oxford, United Kingdom: Oxford University Press.
47. Dunning Hotopp JC, Lin M, Madupu R, Crabtree J, Angiuoli SV, Eisen JA, Seshadri R, Ren Q, Wu M, Utterback TR, Smith S, Lewis M, Khouri H, Zhang C, Niu H, Lin Q, Ohashi N, Zhi N, Nelson W, Brinkac LM, Dodson RJ, Rosovitz MJ, Sundaram J, Daugherty SC, Davidsen T, Durkin AS, Gwinn M, Haft DH, Selengut JD, Sullivan SA, Zafar N, Zhou L, Benahmed F, Forberger H, Halpin R, Mulligan S, Robinson J, White O, Rikihisa Y, Tettelin H, 2006. Comparative genomics of emerging human ehrlichiosis agents. *PLoS Genet 2:* e21.
48. Barbet AF, Lundgren AM, Alleman AR, Stuen S, Björsdorff A, Brown RN, Drazenovich NL, Foley JE, 2006. Structure of the expression site reveals global diversity in MSP2 (P44) variants in *Anaplasma phagocytophilum*. *Infect Immun 74:* 6429–6437.
49. Scharf W, Schauer S, Freyburger F, Petrovec M, Schaarschmidt-Kiener D, Liebisch G, Runge M, Ganter M, Kehl A, Dumler JS, Garcia-Perez AL, Jensen J, Fingerle V, Meli ML, Ensser A, Stuen S, von Loewenich FD, 2011. Distinct host species correlate with *Anaplasma phagocytophilum* ankA gene clusters. *J Clin Microbiol 49:* 790–796.
50. Bown KJ, Lambin X, Ogden NH, Begon M, Telford G, Woldehiwet Z, Birtles RJ, 2009. Delineating *Anaplasma phagocytophilum* ecotypes in coexisting, discrete enzootic cycles. *Emerg Infect Dis 15:* 1948–1954.
51. Portillo A, Perez-Martinez L, Santibanez S, Santibanez P, Palomar AM, Oteo JA, 2011. *Anaplasma spp.* in wild mammals and *Ixodes ricinus* from the North of Spain. *Vector Borne Zoonotic Dis 11:* 3–8.

SUPPLEMENTAL TABLE 1

Variation in *Anaplasma phagocytophilum* sequence from samples obtained from ticks in this study

GenBank accession number with 100% Ident*	Raw sequence between primers†	LE000032001 (*N* = 30)	LE640115007 (*N* = 27)	LE120-11019 (*N* = 25)	LE121-11018 (*N* = 38)	LE200-11013 (*N* = 36)	LE298-11050 (*N* = 39)	LE324-11019 (*N* = 28)	LE433-11011 (*N* = 27)	LE433-11013 (*N* = 38)	LE475-11048 (*N* = 38)
PCR assay result was *Ap*-ha											
Human active-like sequences											
U02521 (human active)	ATAAAGAATAGTT	20	19	15	32	18	19	23	26	29	26
AF136714, AJ242783, FN390880, HQ629914, JN181083, JN181069, JN181081, KC800983	ATGAAGAATAGTT	1	0	0	0	0	0	0	0	2	0
AY035312, EU839849, KC800985	ATAGAGAATAGTT	2	2	1	0	2	2	0	0	2	3
DQ088128	ATAAGGAATAGTT	2	6	2	1	10	4	0	0	1	4
GU391317	GTAAAGAGTAGTT	0	0	1‡	0	0	0	0	0	0	0
JN181066	ATAAAGGATAGTT	0	0	0	3	2	8	0	0	0	0
JN217094	GTAAAGAATAGTT	0	0	1	0	0	0	0	1	0	0
NA	ACAAAGAATAGTT	2	0	1	0	4	6	0	0	1	4
NA	ATAAAAAATAGTT	0	0	0	0	0	0	0	0	0	1
NA	ATAAAGAATGGTT	1‡	0	0	0	0	0	0	0	0	0
NA	ATAAAGAGTAGTT	0	0	4	1	0	0	1	0	3	0
NA	ATAAAGTATAGTT	0	0	0	0	0	0	2‡	0	0	0
NA	ATAACGAATAGTT	1‡	0	0	0	0	0	0	0	0	0
NA	ATAATGGATAGTT	1‡	0	0	0	0	0	0	0	0	0
NA	ATATAGAATAGTT	0	0	0	0	0	0	2‡	0	0	0
NA	TTAAAGAATAGTT	0	0	0	1‡	0	0	0	0	0	0
Variant 1-like sequences											
AY193887 (variant 1)	ATGAAGAATAATT	0	0	0	0	0	0	0	0	0	0
AY281776, AY281777, AY281780, AY281783, AY281788, AY281790, AY281804, AY281806, DQ104443, FJ812390, GU236534, GU236544, GU236566, GU236574, HM480377, HM480381, HM480382, HM480383, JF893931, JX627361, JX627374, JX627374	ACGAAGAATAATT	0	0	0	0	0	0	0	0	0	0
NA	ATGAAGTATAATT	0	0	0	0	0	0	0	0	0	0
NA	ATGAGGAATAATT	0	0	0	0	0	0	0	0	0	0
NA	ATGGAGAATAATT	0	0	0	0	0	0	0	0	0	0
NA	ATGTAGAATAATT	0	0	0	0	0	0	0	0	0	0
Percent correct		66.7	70.4	60	84.2	50	48.7	82.1	96.3	76.3	68.4

(continued)

SUPPLEMENTAL TABLE 1

Continued

GenBank accession number with 100% Ident*	Raw sequence between primers†	LE003709007 (*N* = 37)	LE003709011 (*N* = 40)	LE003709027 (*N* = 40)	LE003709028 (*N* = 39)	LE642055008 (*N* = 38)	LE642055021 (*N* = 39)	LE707015007 (*N* = 40)	LE052-11001 (*N* = 39)	LE184-11015 (*N* = 33)	LE231-11013 (*N* = 31)
PCR assay result was *Ap*-v1											
Human active-like sequences											
U02521 (human-active)	ATAAAGAATAGTT	0	0	0	0	0	0	0	0	0	0
AF136714, AJ242783, FN390880, HQ629914, JN181083, JN181069, JN181081, KC800983	AT*G*AAGAATAGTT	0	0	0	0	0	0	0	0	0	0
AY035312, EU839849, KC800985	ATA*G*AGAATAGTT	0	0	0	0	0	0	0	0	0	0
DQ088128	ATAA*G*GAATAGTT	0	0	0	0	0	0	0	0	0	0
GU391317	*G*TAAAGA*G*TAGTT	0	0	0	0	0	0	0	0	0	0
JN181066	ATAAAG*G*ATAGTT	0	0	0	0	0	0	0	0	0	0
JN217094	*G*TAAAGAATAGTT	0	0	0	0	0	0	0	0	0	0
NA	A*C*AAAGAATAGTT	0	0	0	0	0	0	0	0	0	0
NA	ATAAA*A*AATAGTT	0	0	0	0	0	0	0	0	0	0
NA	ATAAAGAAT*G*GTT	0	0	0	0	0	0	0	0	0	0
NA	ATAAAGA*G*TAGTT	0	0	0	0	0	0	0	0	0	0
NA	ATAAAG*T*ATAGTT	0	0	0	0	0	0	0	0	0	0
NA	ATAA*C*GAATAGTT	0	0	0	0	0	0	0	0	0	0
NA	ATAA*TG*GATAGTT	0	0	0	0	0	0	0	0	0	0
NA	ATA*T*AGAATAGTT	0	0	0	0	0	0	0	0	0	0
NA	*T*TAAAGAATAGTT	0	0	0	0	0	0	0	0	0	0
Variant 1-like sequences											
AY193887 (variant 1)	ATGAAGAATAATT	35	38	39	39	38	39	39	37	33	31
AY281776, AY281777, AY281780, AY281783, AY281788, AY281790, AY281804, AY281806, DQ104443, FJ812390, GU236534, GU236544, GU236566, GU236574, HM480377, HM480381, HM480382, HM480383, JF893931, JX627361, JX627374, JX627374	A*C*GAAGAATAATT	0	1‡	0	0	0	0	0	0	0	0
NA	ATGAAG*T*ATAATT	0	1	0	0	0	0	0	1	0	0
NA	ATGA*G*GAATAATT	2	0	1	0	0	0	1	1	0	0
NA	ATG*G*AGAATAATT	0	0	0	0	0	0	0	0	0	0
NA	ATG*T*AGAATAATT	0	0	0	0	0	0	0	0	0	0
Percent correct		94.6	95	97.5	100	100	100	97.5	94.9	100	100

Ap-ha = *Anaplasma phagocytophilum* human-active; *Ap*-v1 = *Anaplasma phagocytophilum* variant 1; NA = not applicable.
*Ident is the percent identity of the query subject alignments in Basic Local Alignment Search Tool (BLAST), and the highest Ident found was 98%.
†Nucleotides differing from human-active strain or variant 1 strain are italicized.
‡This sequence was found in only one of our samples.