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ORIGINAL ARTICLE

Effects of Tick Control by Acaricide Self-Treatment of White-Tailed Deer on Host-Seeking Tick Infection Prevalence and Entomologic Risk for *Ixodes scapularis*-Borne Pathogens

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Abstract

We evaluated the effects of tick control by acaricide self-treatment of white-tailed deer on the infection prevalence and entomologic risk for three *Ixodes scapularis*-borne bacteria in host-seeking ticks. Ticks were collected from vegetation in areas treated with the "4-Poster" device and from control areas over a 6-year period in five geographically diverse study locations in the Northeastern United States and tested for infection with two known agents of human disease, *Borrelia burgdorferi* and *Anaplasma phagocytophilum*, and for a novel relapsing fever-group spirochete related to *Borrelia miyamotoi*. Overall, 38.2% of adults and 12.5% of nymphs were infected with *B. burgdorferi*; 8.5% of adults and 4.2% of nymphs were infected with *A. phagocytophilum*; and 1.9% of adults and 0.8% of nymphs were infected with *B. miyamotoi*. In most cases, treatment with the 4-Poster device was not associated with changes in the prevalence of infection with any of these three microorganisms among nymphal or adult ticks. However, the density of nymphs infected with *B. burgdorferi*, and consequently the entomologic risk for Lyme disease, was reduced overall by 68% in treated areas compared to control areas among the five study sites at the end of the study. The frequency of bacterial coinfections in ticks was generally equal to the product of the proportion of ticks infected with a single bacterium, indicating that enzootic maintenance of these pathogens is independent. We conclude that controlling ticks on deer by self-application of acaricide results in an overall decrease in the human risk for exposure to these three bacterial agents, which is due solely to a reduction in tick density.

Key Words: Acaricide—4-Poster—Entomologic risk index—*Ixodes scapularis*—*Borrelia burgdorferi*—*Anaplasma phagocytophilum*—*Borrelia miyamotoi*—Tick control—White-tailed deer—Coinfection.

Introduction

Ixodes scapularis is the principal North American vector of several human pathogens, among which are *Borrelia burgdorferi*, the spirochetal agent of Lyme disease (Burgdorfer et al. 1982); *Anaplasma phagocytophilum*, the rickettsial agent of human granulocytic anaplasmosis (ehrlichiosis) (Bakken et al. 1994); and a sporozoan parasite, *Babesia microti* (Spielman 1976). A second *Borrelia* species, closely related to the relapsing fever group spirochete *B. miyamotoi* and hereafter referred to as *B. miyamotoi* sensu lato (s.l.), has also been identified in *I. scapularis* (Scoles et al. 2001).

B. burgdorferi is responsible for approximately 20,000 reported cases of Lyme disease each year in the United States, and the annual incidence of Lyme disease continues to increase (CDC 2004). Human granulocytic anaplasmosis is less

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common, with only a few hundred cases reported per year (McNabb et al. 2007), but is a potentially fatal disease (Bakken et al. 1994). Babesiosis, also a potentially fatal disease, occurs only in localized areas of the coastal Northeast and upper Midwest United States. The disease-causing potential of *B. miyamotoi* s.l. in humans remains unclear.

Human risk for exposure to vector-borne pathogens is most accurately assessed by determining entomologic risk (ER, sometimes referred to as an index, ERI), which is calculated as the product of the density of host-seeking vectors and their infection prevalence (Mather et al. 1996). In the case of *I. scapularis*-borne pathogens, nymphal ticks are the primary vectors due to their summer peak in host-seeking activity, which coincides with most human activity outdoors (Fish 1993), and due to their small size, which allows them to evade detection long enough for transmission to occur (Falco et al. 1996, Piesman et al. 1987). Therefore, the ER for *I. scapularis*-borne pathogens can be estimated as the density of infected nymphs. The Northeast Area-wide Tick Control Project (NEATCP), aimed at evaluating the effect of self-application of acaricide to deer on tick control, has shown that the use of "4-Poster" acaricide application devices is highly effective at reducing the density of host-seeking ticks (Brei et al. 2008). In this study, we present the results of the first evaluation of the impact of the 4-Poster device on pathogen infection prevalence in host-seeking *I. scapularis* ticks and on ER as measured by the density of infected nymphs.

Materials and Methods

Tick collections and infection prevalence

Nymphal and adult ticks were collected by either drag sampling or flagging in each of five NEATCP study locations as described in this issue (Carroll et al. 2009, Daniels et al. 2008, Miller et al. 2008, Schulze et al. 2008, Stafford et al. 2008). Tick counts were standardized by sampling effort and were seasonally adjusted as described previously (Brei et al. 2008). Ticks submitted from each of the 5 study locations were randomly selected and then tested for infection with *B. burgdorferi*, *A. phagocytophilum*, and *B. miyamotoi* s.l. at the Yale Vector Ecology Laboratory. Ticks were not assayed for infection with *B. microti*.

Individual ticks were frozen with liquid nitrogen, ground with a DNA-free pestle, and resuspended in 50 μL Tris-EDTA buffer. DNA was extracted using the IsoQuick Nucleic Acid Extraction Kit (Orca Research Inc., Bothell, WA). Tick extracts were aliquoted and screened for the presence of DNA from *B. burgdorferi, A. phagocytophilum*, and *B. miyamotoi* s.l. by separate polymerase chain reaction (PCR) amplifications using previously described primers. *B. burgdorferi* DNA was detected by nested PCR amplification of a 941-bp region of the *B. burgdorferi* 16S–23S rDNA spacer region using nested PCR primers reported by Liveris et al. (1996, 1999). First round primers were P_A (5′-GGTATGTT TAGTGAGGG-3′) and P_{95} (5′-GGTTAGAGCGCAGGTCTG-3′) and second round primers were P_B (5′-CGTACTG GAAAGTGCGGCTG-3′) and P_{97} (5′-GATGTTCAACTCAT CCTGGTCCC-3′). Reaction conditions for both rounds were 35 cycles of denaturation at 94°C for 30 sec, annealing at 52°C for 30 sec, and extension at 72°C for 30 sec. Samples were screened for the presence of *A. phagocytophilum* DNA using primers specifically targeting its 16S rDNA that were previously described by Massung and Slater (2003). Primer sequences were EHR521 (5′-TGTAGGCGGTTCGGTAAGTTA AAG-3′) and EHR747 (5′-GCACTCATCGTTTACAGCGTG-3′). Reaction conditions consisted of 50 rounds of denaturation at 94°C for 30 sec, annealing at 55°C for 20 sec, and extension at 72°C for 1 min. It should be noted that our PCR assay did not distinguish between different genotypes of *A. phagocytophilum*, of which one variant is consistently associated with human disease (Massung et al. 2002) while others may only infect deer (Belongia et al. 1997, Massung et al. 1998).

Testing for the novel *B. miyamotoi* s.l. was done using primers that specifically amplify a 219-bp fragment of its flagellin gene and were first reported by Scoles et al. (2001). Primers were FLA181F (5′-CCAGCATCATTAGCTGGAA-3′) and FLA400R (5′-CACCTTGAACTGGAGCGGCT-3′). Reaction conditions consisted of 35 rounds of denaturation at 94°C for 30 sec, annealing at 62°C for 30 sec, and extension at 72°C for 30 sec. All reactions were performed using PCR SuperMix (Invitrogen, Carlsbad, CA) with 0.2 μM of both the forward and the reverse primers and were accompanied by positive and negative controls. All PCR products were visualized on a 2% ethidium bromide agarose gel.

Statistical analyses

We used logistic regression to evaluate the effects of treatment with the 4-Poster device on the proportions of ticks infected with the three bacterial agents. Variables for study site and year were included as covariates in the models in order to adjust for potential confounding due to site and year effects. All odds ratios (ORs) are expressed with reference to control sites. Analysis of variance (ANOVA) was used to evaluate the effect of treatment with the 4-Poster device on ER as measured by the density of infected nymphs, adjusting for the effects of study location, dates of sampling, and year. The density of infected nymphs was log-transformed for all ANOVA calculations in order to achieve normality according to the Shapiro-Wilk W test. Site-specific differences in infection prevalence between treatment and control areas were evaluated by logistic regression, with year included as a covariate to adjust for overall differences in infection prevalence by year. A Bonferroni correction was applied to multiple comparisons. Temporal trends in infection prevalence and the density of infected nymphs were investigated using linear regression with adjustments made for any effects of site.

The null hypothesis of independent infection among ticks by *B. burgdorferi, A. phagocytophilum*, and *B. miyamotoi* s.l. was tested by comparing the observed and expected frequencies of single and mixed infections. Pearson's χ^2 test was used for coinfection of *B. burgdorferi* and *A. phagocytophilum*, where all observed and expected frequencies were $\geq$5, and Fisher exact test was used for other coinfections, where observed or expected frequencies were $<$5. All statistical calculations were performed using STATA/SE 10 (StataCorp, College Station, TX).

Results

Prevalence of infection with B. burgdorferi, A. phagocytophilum, and B. miyamotoi s.l.

Over the 6 years of the NEATCP study, 1,828 adult and 4,276 nymphal *I. scapularis* ticks were examined for infection

Table 1. Nymphal and Adult *I. scapularis* Infection Prevalence and Density of Infected Nymphs (Entomologic Risk) in Treatment and Control Areas for *B. burgdorferi, A. phagocytophilum,* and *B. miyamotoi* s.l.

			Proportion of ticks infected					*DIN*			
Infection	I. scapularis *stage*	*4-Poster area type*	n *positive (%)*	*Crude OR (95% CI)*	p	*Adjusted OR (95% CI)*	p	*DIN (per 1000 m^2)*	F	*df*	p
B. burgdorferi	Adult	Control	360 (37.7)								
		Treatment	340 (38.9)	1.03 (0.85, 1.25)	0.61	0.93 (0.76, 1.13)	0.49	—			
B. burgdorferi	Nymph	Control	246 (11.8)					3.65			
		Treatment	287 (13.1)	1.11 (0.93, 1.33)	0.18	1.08 (0.89, 1.29)	0.44	2.10	9.49	1	0.004
A. phagocytophilum	Adult	Control	61 (6.7)								
		Treatment	85 (10.5)	1.64 (1.17, 2.33)	0.01	1.70 (1.19, 2.43)	0.003	—			
A. phagocytophilum	Nymph	Control	96 (4.2)					1.55			
		Treatment	98 (4.1)	0.95 (0.71, 1.27)	0.77	0.85 (0.63, 1.14)	0.28	0.75	5.88	1	0.02
B. miyamotoi s.l.	Adult	Control	13 (2.1)								
		Treatment	9 (1.6)	0.81 (0.35, 1.94)	0.55	0.83 (0.33, 2.05)	0.68	—			
B. miyamotoi s.l.	Nymph	Control	5 (0.6)					0.40			
		Treatment	9 (1)	1.78 (0.59, 5.34)	0.32	1.70 (0.56, 5.13)	0.35	0.15	0.07	1	0.84

OR, odds ratio; CI, confidence interval; DIN, density of infected nymphs.

with *B. burgdorferi*; 1,712 adults and 4,674 nymphs for infection with *A. phagocytophilum*; and 1,163 adults and 1,709 nymphs for infection with *B. miyamotoi* s.l. Overall, 38.2% of adults and 12.5% of nymphs were infected with *B. burgdorferi*; 8.5% of adults and 4.2% of nymphs were infected with *A. phagocytophilum*; and 1.9% of adults and 0.8% of nymphs were infected with *B. miyamotoi* s.l. The proportions of ticks infected with each of the three agents in NEATCP treatment and control areas are shown in Table 1. After adjusting for differences in tick sampling effort or success among the different sites and years of the study, no relationship was found between infection prevalence and acaricide treatment, except in the case of adult ticks infected with *A. phagocytophilum* (adjusted OR, 1.70; 95% confidence interval [CI], 1.19–2.43; $p = 0.003$) (Table 1), where there was a significantly higher infection prevalence in the treatment areas compared with control areas.

Comparisons of treatment and control areas by site revealed substantial between-site variation in infection prevalence for all three agents (Fig. 1). When broken down by site, and after adjusting for overall differences between different years of the study, there was a significantly higher proportion of nymphs infected with *B. burgdorferi* in treatment areas compared with control areas in New York (adjusted OR, 2.08; 95% CI, 1.33–3.25; corrected $p = 0.005$). We also found a significantly higher prevalence of *A. phagocytophilum* in treatment areas compared with control areas in nymphs from Maryland (adjusted OR, 9.89; 95% CI, 2.66–36.78; corrected $p = 0.005$) and in adults from New Jersey (adjusted OR, 2.57; 95% CI, 1.30–5.11; corrected $p = 0.04$).

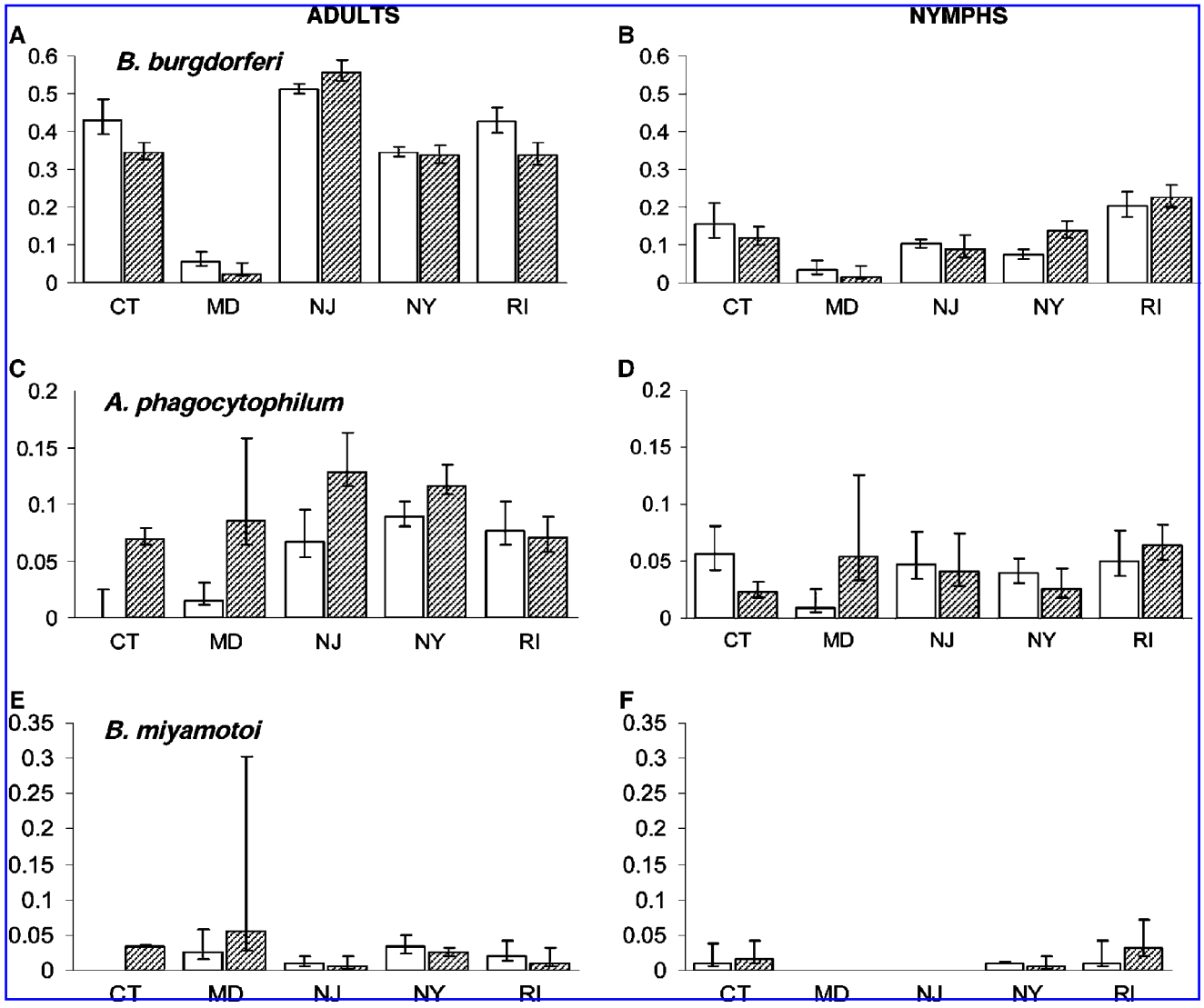

FIG. 1. Prevalence of *Borrelia burgdorferi* and *Anaplasma phagocytophilum* infection in host-seeking nymphal and adult ticks, by study site. Prevalence of infection with *B. burgdorferi, A. phagocytophilum*, or *Borrelia miyamotoi* s.l. by site in adults and nymphs in control (open bars) and treatment (hatched bars) areas. Error bars represent ± standard error. (**A**) Adults infected with *B. burgdorferi*; (**B**) nymphs infected with *B. burgdorferi*; (**C**) adults infected with *A. phagocytophilum*; (**D**) nymphs infected with *A. phagocytophilum*; (**E**) adults infected with *B. miyamotoi* s.l.; (**F**) nymphs infected with *B. miyamotoi* s.l.

There were no significant changes in infection prevalence over time for *B. burgdorferi* or for *A. phagocytophilum* (Fig. 2) in either treatment or control areas; however, there was a statistically nonsignificant increase in *B. burgdorferi* infection prevalence in adult ticks in both treatment and control areas. *B. miyamotoi* s.l. was omitted from temporal analyses because testing was only performed on samples collected in the final 2 years of the study.

ER for infection with B. burgdorferi, A. phagocytophilum, *and* B. miyamotoi *s.l.*

Using ANOVA and adjusting for study location, sampling dates, and year, we observed significant overall reductions in the density of infected nymphs, or ER, for both *B. burgdorferi* and *A. phagocytophilum* and a nonsignificant reduction for *B. miyamotoi* s.l., between treatment and control areas with ER being lower in treatment areas (Table 1).

After adjusting for the effects of study location, analysis of temporal trends revealed significant declines in the ER for *B. burgdorferi* over the 6 years of the study in treatment areas ($R^2 = 0.53$, $F(5, 11) = 4.04$; $p = 0.002$), but not in control areas, where, with the exception of an increase in 2000, the density of infected nymphs stayed relatively stable over time ($R^2 = 0.44$, $F(5, 18) = 2.79$; $p = 0.26$) (Fig. 3). By the final year of the study, ER for *B. burgdorferi* had declined overall by 68% in treatment areas relative to control areas. Similarly, there was a significant decline in the ER over time for *A. phagocytophilum* in treatment areas ($R^2 = 0.59$, $F(5, 15) = 4.23$; $p = 0.023$), but not in control areas ($R^2 = 0.51$, $F(5, 13) = 2.76$; $p = 0.32$).

Mixed infections

Mixed infections occurred in approximately the same proportion as would be expected under the null hypothesis that the prevalence of coinfection with two organisms is the product of the prevalence of the two infections individually, with one exception: mixed *B. burgdorferi* and *B. miyamotoi* s.l. occurred less frequently than would be expected (Fisher exact test, $p = 0.003$) (Table 2).

Discussion

The success of vector control programs for reducing human disease risk should be measured by effects on ER, which takes into account both the density of host-seeking vectors in the environment and their infection prevalence. However, only a minority of evaluations of tick control programs has included infection prevalence in their assessments. Notably

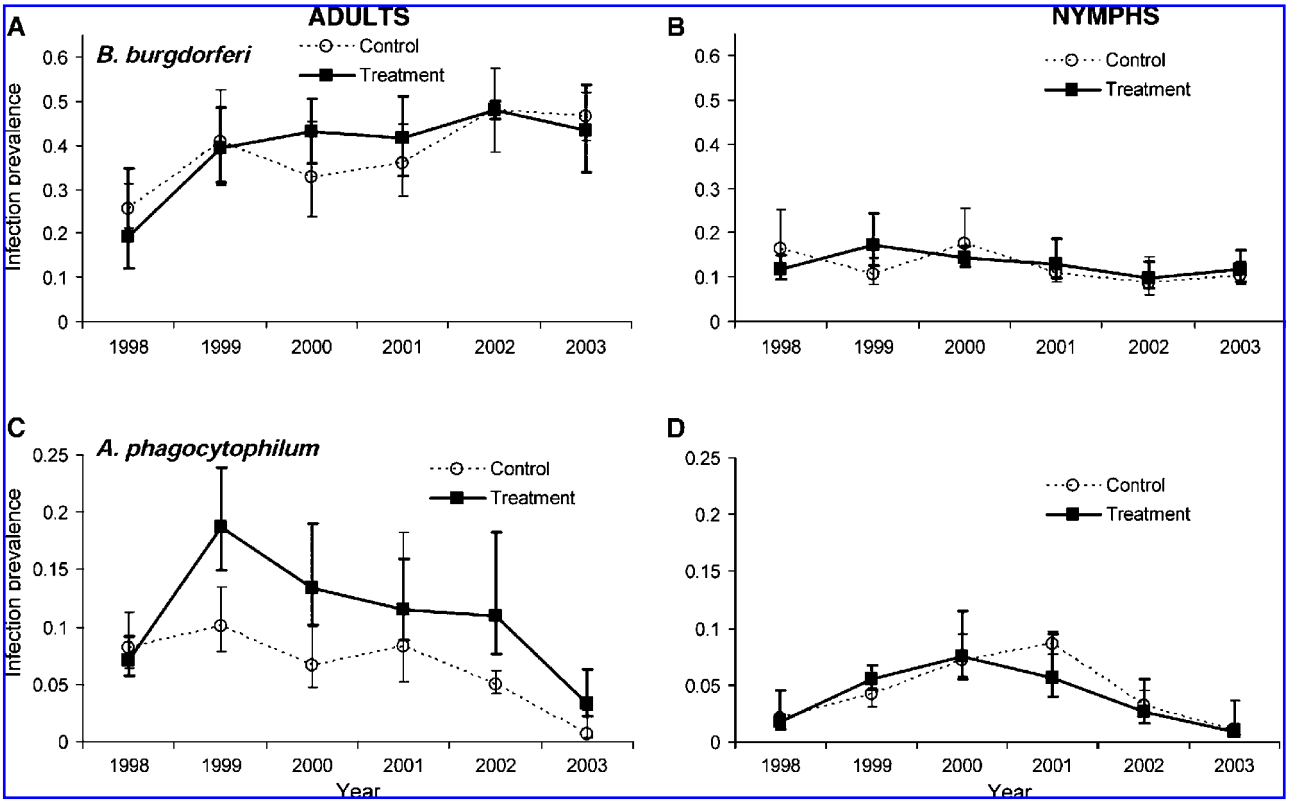

FIG. 2. Host-seeking nymphal and adult *Ixodes scapularis* infection prevalence. Prevalence of infection with *Borrelia burgdorferi* and *Anaplasma phagocytophilum* infection in host-seeking nymphal and adult ticks over the 6 years of the study. (**A**) Prevalence of infection with *B. burgdorferi* in adult *I. scapularis* over time in treatment (solid line) and control (dashed line) areas. (**B**) Prevalence of infection with *B. burgdorferi* in nymphal *I. scapularis* over time in treatment (solid line) and control (dashed line) areas. (**C**) Prevalence of infection with *A. phagocytophilum* in adult *I. scapularis* over time in treatment (solid line) and control (dashed line) areas. (**D**) Prevalence of infection with *A. phagocytophilum* in nymphal *I. scapularis* over time in treatment (solid line) and control (dashed line) areas. *B. miyamotoi* s.l. was not analyzed temporally because testing was only preformed performed on samples collected in the final 2 years of the study. Error bars represent ± standard error.

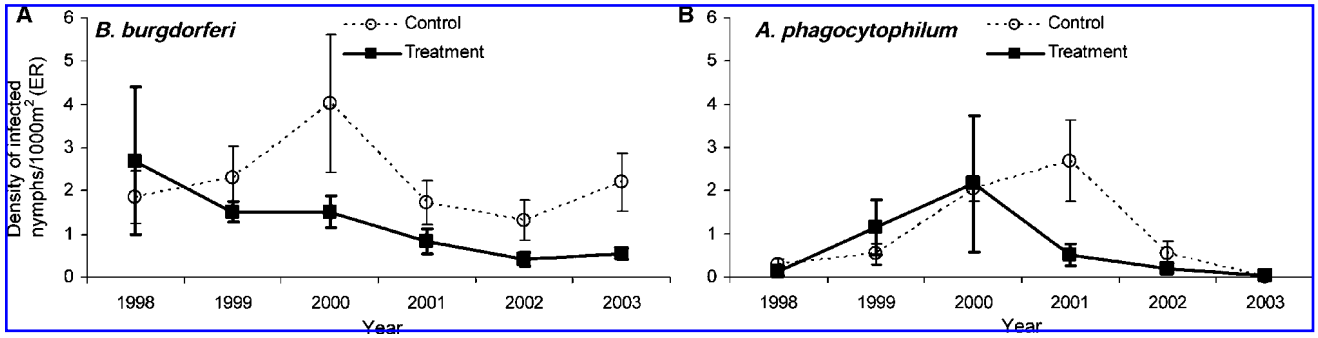

FIG. 3. Entomologic risk as measured by the density of nymphs infected with *Borrelia burgdorferi* and *Anaplasma phagocytophilum* over the 6 years of the study in treatment and control areas. (**A**) Density of nymphs (per 1000 m^2) infected and with *B. burgdorferi* over time in treatment (solid line) and control (dashed line) areas. (**B**) Density of nymphs (per 1000 m^2) infected with *A. phagocytophilum* over time in treatment (solid line) and control (dashed line) areas. *Borrelia miyamotoi* s.l. were not analyzed temporally because testing was only preformed performed on samples collected in the final 2 years of the study. Error bars represent ± standard error.

among these, one study found a sharp, but temporary, increase in *B. burgdorferi* infection rates in host-seeking *I. scapularis* ticks upon removal of white-tailed deer from an offshore Maine island (Rand et al. 2004). The authors' explanation of this result was that the dramatic decline in the deer population forced the remaining immature ticks, many of which would have fed on reservoir-incompetent deer, to feed exclusively on reservoir-competent rodents. Therefore, a major concern of tick control methods that target reservoir-incompetent hosts should be the possibility of a shift in feeding of immature ticks to reservoir-competent species. However, because the NEATCP aimed to kill adult ticks feeding on deer, rather than reduce deer density, we expected that this approach would have no effect on host-seeking tick infection prevalence.

In this study, reducing the density of host-seeking ticks by self-application of acaricide to white-tailed deer was not associated with changes in the prevalence of three bacterial infections among host-seeking ticks, with the exception of a significant increase in the prevalence of *A. phagocytophilum* in adult ticks. However, overall, treatment with the 4-Poster device significantly reduced the density of host-seeking nymphs with the consequence of significantly reducing the density of infected ticks, or ER (Table 1).

Over the 6 years of the study, the proportion of nymphs infected with *B. burgdorferi* remained relatively stable in both treatment and control areas (Fig. 2B). In contrast, ER for *B. burgdorferi* declined steadily over time from the first year of the study in treatment areas, while in control areas the density of infected nymphs was variable but exhibited no significant change over time (Fig. 3A). By the final year of the study, the average reduction in ER for *B. burgdorferi* in treatment sites compared with control sites was 68%, with all study locations achieving at least an 84% reduction in ER with the exception of New York, which only achieved a 24% reduction in ER. The reason for the smaller decline in New York is unclear; however, it seems to be due to the higher nymphal infection prevalence in the treatment area of that study location compared with control area rather than a failure of the 4-poster device to reduce tick densities. However,

Table 2. Observed Proportions of Ticks with Two Infections Compared with Expected Coinfections Based on Single Infection Prevalence Estimates

Stage	*Combination*	*Single infections (% of total ticks tested for both infections)*	*Prevalence of mixed infections (%)*		χ^2	*df*	p
			Expected	*Observed*			
Adults	*B. burgdorferi* with	587 (38.72)					
	A. phagocytophilum	131 (8.64)	3.35	3.23	0.1	1	0.75
Nymphs	*B. burgdorferi* with	400 (11.73)					
	A. phagocytophilum	145 (4.25)	0.50	0.38	1.12	1	0.29
Adults	*B. burgdorferi* with	458 (39.79)					
	B. miyamotoi s.l.	21 (1.82)	0.73	0.17	N/A		0.003
Nymphs	*B. burgdorferi* with	164 (9.89)					
	B. miyamotoi s.l.	14 (0.84)	0.08	0.06	N/A		1
Adults	*A. phagocytophilum* with	83 (7.50)					
	B. miyamotoi s.l.	20 (1.81)	0.14	0.00	N/A		0.39
Nymphs	*A. phagocytophilum* with	33 (1.93)					
	B. miyamotoi s.l.	14 (0.82)	0.02	12	N/A		0.22

temporal trends in the prevalence of nymphs infected with *B. burgdorferi* in this study location are similar (data not shown), suggesting that the elevated infection prevalence in the treatment area is a consequence of some characteristic of the study location and not a result of the use of the 4-poster device.

The ER for *A. phagocytophilum* declined in treatment areas relative to control sites; however, these declines were not monotonic (Fig. 3B). Declines in the density of nymphs infected with *A. phagocytophilum* were preceded by increased densities of infected ticks during the first 3 and 4 years of the study in treatment and control areas, respectively. This temporal fluctuation apparently represents natural temporal variability in the prevalence of *A. phagocytophilum*-infected nymphs (Fig. 2D) and was unrelated to nymphal density. By the final year of the study, the overall ER for *A. phagocytophilum* was approaching zero in both treatment and control areas due to very a low prevalence across all study locations that year. However, in 2001 and 2002, there were average reductions in ER of 76% and 70%, respectively, and no study location achieved less than a 47% reduction in either year (data not shown).

Coinfection with multiple pathogens has been previously observed in both host-seeking *I. scapularis* ticks and human patients (Nadelman et al. 1997, Schwartz et al. 1997). Coinfection with *B. burgdorferi* and *A. phagocytophilum* has important epidemiologic and clinical implications because these pathogens can cause diseases characterized by a variety of overlapping nonspecific symptoms, which can complicate diagnosis. We found the number of mixed infections with *B. burgdorferi* and *A. phagocytophilum* to be consistent with the number expected under a null hypothesis of independent pathogen maintenance and acquisition. The large number of field-derived ticks examined for mixed *B. burgdorferi* and *A. phagocytophilum* infection ($n = 4926$) in this study support the previous conclusion that these pathogens are independently maintained in nature (Daniels et al. 1998, Schwartz et al. 1997), and the probability of encountering a coinfected tick is equal to the product of the probability of encountering two ticks infected with different pathogens. The frequency of mixed infections of adults with the *B. burgdorferi* and *B. miyamotoi* s.l. was significantly lower than expected given the frequencies of single infections (Table 2). One possible explanation for this is that these two pathogens do not share the same range of reservoir hosts. *B. burgdorferi* is known to infect a wide range of warm-blooded mammals and birds (Anderson et al. 1986, Fish and Daniels 1990), but the host range of the novel *B. miyamotoi* s.l. is not yet known.

The diverse natural forces that govern the dynamics and parameters of enzootic transmission of tick-borne pathogens are not completely understood and represent an area of active research. It is important, therefore, to empirically evaluate the effects of any intervention on these parameters. This evaluation of the NACTP has shown that reducing tick burdens on white-tailed deer by acaricide self-treatment can effectively reduce the densities of nymphs infected with *B. burgdorferi* and *A. phagocytophilum*, the determinants of risk for Lyme disease and human granulocytic anaplasmosis, respectively, in endemic areas of the Northeast United States. Importantly, the nature of the tick control achieved by this treatment does not alter the vertebrate host diversity available to host-seeking ticks, which can result in unanticipated temporary increases in human risk for Lyme disease (Rand et al. 2004). This study demonstrates that the 4-Poster device is an effective disease risk management tool that can be used in endemic areas to prevent multiple tick-borne infections in humans and domestic animals by reducing ER in a significant, yet predictable manner.

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Disclosure Statement

No conflicts to declare.

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