Elimination of *Borrelia burgdorferi* and *Anaplasma phagocytophilum* in Rodent Reservoirs and *Ixodes scapularis* Ticks Using a Doxycycline Hyclate-Laden Bait

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Abstract. A field trial was conducted in a Lyme disease-endemic area of New Jersey to determine the efficacy of a doxycycline hyclate rodent bait to prophylactically protect and cure small-mammal reservoirs and reduce infection rates in questing *Ixodes scapularis* ticks for *Borrelia burgdorferi* and *Anaplasma phagocytophilum*. The doxycycline-laden bait was formulated at a concentration of 500 mg/kg and delivered during the immature tick feeding season in rodent-targeted bait boxes. The percentage of infected small mammals recovered from treated areas after 2 years of treatment was reduced by 86.9% for *B. burgdorferi* and 74% for *A. phagocytophilum*. Infection rates in questing nymphal ticks for both *B. burgdorferi* and *A. phagocytophilum* were reduced by 94.3% and 92%, respectively. Results from this study indicate that doxycycline-impregnated bait is an effective means of reducing infection rates for *B. burgdorferi* and *A. phagocytophilum* in both rodent reservoirs and questing *I. scapularis* ticks.

INTRODUCTION

Despite continued increases in the incidence of disease resulting from pathogens transmitted by *Ixodes scapularis* Say, including Lyme disease, human babesiosis, and human granulocytic anaplasmosis, in many parts of the United States, successful prevention efforts have not kept pace.¹ As a result, the development of reliable interventions to reduce tick-borne disease risk remains a public health priority.^{2,3}

Reducing vector tick abundance is among the most effective ways of mitigating the risk of disease transmission.^{3,4} Area-wide application of chemical acaricides is perhaps the most widely investigated, rapid, and reliable means of suppressing *I. scapularis*.^{2,5,6} However, the use of habitat-targeted acaricides is generally viewed by the public as having adverse health effects and undesirable environmental impacts.^{7,8} Consequently, it has been estimated that < 25% of households in Lyme disease-endemic communities treat their properties to control ticks.^{9,10} Host-targeted chemical control provides an alternative to area-wide acaricide applications. The 4-Poster (ARS, USDA, Kerrville, TX) topical treatment device, which targets adult *I. scapularis* parasitizing white-tailed deer (*Odocoileus virginianus*), and the Maxforce TMS (Tick Box Technology Corp., Norwalk, CT) bait boxes, designed to treat a variety of small-mammal hosts of immature *I. scapularis*, have been shown to be effective when used alone or in combination.^{11–13} Although these technologies have the advantage of minimizing the amount of acaricide introduced into the environment, their widespread use has been constrained by regulatory and economic considerations.^{1,12}

We report a novel host-targeted approach using doxycycline hyclate-impregnated bait.^{14–16} After promising laboratory studies, we conducted a field trial in a Lyme disease-endemic area in central New Jersey to assess the ability of these baits to prophylactically protect small-mammal reservoirs, cure infected reservoirs, and reduce infection of immature *I. scapularis* ticks by both the Lyme disease spirochete, *Borrelia burgdorferi*, and the agent of human granulocytic anaplasmosis, *Anaplasma phagocytophilum*.

MATERIALS AND METHODS

Study areas. The study was conducted in an area of mixed hardwood forest located in Millstone Township, Monmouth County, New Jersey, where *I. scapularis* and its small-mammal hosts are well-established.^{12,17,18} The 10- to 15-m canopy was dominated by chestnut oak (*Quercus prinus* L.), red oak (*Q. rubra* L.), and white oak (*Q. alba* L.), with associated species including red maple (*Acer rubrum* L.), yellow poplar (*Liriodendron tulipifera* L.), black gum (*Nyssa sylvatica* Marsh.), and American beech (*Fagus grandifolia* Ehrh.). The understory and shrub layer consisted of saplings and seedlings of canopy species, highbush blueberry (*Vaccinium corymbosum* L.), lowbush blueberry (*V. angustifolium* Ait.), huckleberries (*Gaylussacia* spp.), sweet pepperbush (*Clethra alnifolia* L.), and greenbriar (*Smilax glauca* Walt.). The treatment site and one of the control sites were ~2.5-ha single-family residential properties located ~200 m apart. A second undeveloped control site (~3 ha) was located ~1,000 m south in nearby Assunpink Wildlife Management Area (WMA).

Deployment and maintenance of bait stations. Beginning in May of 2007 and 2008, 50 Protecta LP bait stations (Bell Laboratories, Inc., Madison, WI) were fitted with ~135 g extruded, wax-based bait blocks containing 500 mg/kg doxycycline (Genesis Laboratories, Inc., Wellington, CO) and deployed at the treatment site. Bait stations were placed in forested habitat at ~20-m intervals along two concentric perimeters at distances of ~5 and 25 m from the lawn-forest interface. Whenever possible, bait stations were deployed adjacent to natural structures, including fallen trees, stumps, brush piles, woodpiles, and outbuildings, which are considered to be likely foraging or nesting sites for small mammals. Bait stations were inspected weekly to assess overall condition and bait consumption, and they were rebaited as necessary. Consumption of bait was recorded using a five-point Activity Index (1 = no bait consumption, 2 = 25% bait consumed, 3 = 50% bait consumed, 4 = 75% bait consumed, and 5 = 100% bait consumed) (Figure 1). In addition, any bait station that was significantly damaged by eastern gray squirrels (*Sciurus carolinensis*) was replaced. All bait stations were withdrawn in September of both years after 19 consecutive weeks of deployment.

Small-mammal trapping and tick burdens. Small mammals were collected using 7.6 × 8.9 × 30.5-cm Sherman non-folding

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^aBait boxes were maintained weekly for a total of 19 wk.

^bThe total for each week represents a 2 year avg of bait box use (May 2007 – Sept 2008)

FIGURE 1. Summary of doxycycline bait station use as measured by estimates of bait consumption. Bait consumption was based on an activity index: 1 = no activity, 2 = 25% bait consumed, 3 = 50% bait consumed, 4 = 75% bait consumed, and 5 = 100% bait consumed. Bait stations were deployed from June to September for a total of 19 wk in 2007 and 2008.

box traps (H. B. Sherman, Tallahassee, FL) baited with rolled oats for food and cotton balls for nesting material and 40.6 × 12.7 × 12.7-cm Tomahawk Model 102 rigid single-door live traps (Tomahawk Traps and Equipment, Tomahawk, WI) baited with apple slices. Pre-intervention small-mammal and tick burden data were obtained from a single trapping event conducted during May of 2007, whereas post-intervention small-mammal and tick burden data were collected during the months of June and August of 2007 and 2008, the peak activity periods of *I. scapularis* nymphs and larvae, respectively.^{12,18,19} Post-intervention trapping provided data on the (1) species composition of the small-mammal community in treated and untreated areas, (2) tick burdens, (3) prevalence of infection with *B. burgdorferi* and *A. phagocytophilum* in small mammals and attached ticks in treated and untreated sites, and (4) blood plasma levels of doxycycline in sampled small mammals. During each trapping event, 100 Sherman traps and 10 Tomahawk traps were set at each site for 3 consecutive days. All traps were deployed by mid-afternoon and checked by mid-morning on the following day. Traps remained open during the day and were checked periodically until late afternoon. Captured rodents were transported to a central processing location and anesthetized with isoflurane. Ectoparasites were removed with fine forceps and placed in 70% ethanol for species identification and evaluation for infection with *B. burgdorferi* and *A. phagocytophilum* by polymerase chain reaction (PCR). Ear biopsies were taken, surface-sterilized, and cultured in Barbour-Stoner-Kelley (BSK-H) media for isolation of *B. burgdorferi*.²⁰ Cultures were read by dark-field microscopy every 7 days for 4 weeks before negative cultures were discarded.²¹ In addition, ~300 µL ethylenediaminetetraacetic acid (EDTA) blood were obtained from each capture and placed in microtainers (Becton Dickinson, Franklin Lakes, NJ). Serum was subsequently used to determine pharmacokinetic levels of doxycycline hyclate in small-mammal plasma by high-pressure liquid chromatography (HPLC).¹⁵ Animals were ear-tagged (Monel Model 1005-1 or 1005-3; National Band and Tag Company, Newport, KY) and weighed, their overall body measurements were recorded (total body length, tail length, right ear length, and right hind foot length), and subsequently, they were

released at the point of capture.¹¹ Small mammals recaptured during a particular trapping event were not reprocessed.

Animals were handled according to approved protocols on file with the Centers for Disease Control and Prevention, Division of Vector-Borne Diseases Animal Care and Use Committee Protocol 08-008.

Tick collections. Host-seeking ticks were collected using a combination of dragging and walking methods.^{22,23} Each site was sampled until a minimum of 50 ticks was collected. In 2007, sampling for subadult *I. scapularis* was performed to coincide with trapping events. Thereafter, adults were collected during the first week of November of 2007, April of 2008, and November of 2009, whereas nymphs were obtained in June of 2008 and 2009. All sampling was performed between 08:00 and 12:00 hours, when vegetation was dry and wind was judged to be below 10 km/hour.^{24,25} Ticks adhering to drags and coveralls were removed and placed into discrete vials containing 70% ethanol for later analyses by PCR.

Analysis of ticks and small-mammal blood for *B. burgdorferi* and *A. phagocytophilum* infection. DNA was extracted from ticks using the 1.5-mL Kontes disposable pestle system (Kimble Chase, Vineland, NJ) and the DNeasy Blood and Tissue kit (Qiagen, Valencia, CA). Briefly, individual ticks were homogenized using a pestle and 180 µL buffer ATL plus 20 µL proteinase K in a 1.5-mL microfuge tube; the solution was incubated overnight at 56°C. Approximately 12 hours later, 200 µL buffer AL were added to each sample, and the samples were vortexed and incubated at 70°C for 10 minutes. Subsequently, 250 µL ethanol (100%) were added, and the samples were vortexed thoroughly. The samples were then loaded onto a Qiagen DNeasy column and washed with buffer AW1 and AW2 according to the manufacturer's protocol. Finally, the columns were eluted with 50 µL buffer AE. To ensure the presence of DNA, all tick extracts were tested for tick actin DNA by real-time PCR (qPCR). The primers used for the actin control PCR were actin-F 5'-CCATCCAGGCCGTGCTCTC-3' and actin-R 5'-ATCTTCATCAGGTAGTCGGTCAGG-3'. The actin qPCR was done in FastStart CYBR Green Master mix (Roche, Indianapolis, IN) with 1 µM each primer and 2 µL sample in a 25-µL reaction. The samples, including a positive

sample and a water control, were analyzed in a 96-well format with the following parameters: 1 cycle at 95°C for 10 minutes and 40 cycles at 95°C for 10 seconds, 60°C for 20 seconds, and 72° for 30 seconds, and recording at 82°C using a real-time detection system (Bio-Rad Laboratories, Hercules, CA). At the end of each run, a melting curve analysis was performed to verify the correct amplicon. Crossing threshold (C_T) and melting curves were determined using Opticon Monitor 3 software. Only samples that were positive for tick actin were included in this study. To verify the presence of *B. burgdorferi* s.s. in a sample, we performed qPCR amplifying the *fliD* locus. The primers used in this TaqMan probe assay were *fliD*-F 5'-TGGTGACAGAGTGATGATAATGGAA-3', *fliD*-R 5'-ACTCCTCCGGAAGCCACAA-3', and *fliD*-probe 5'-FAM-TGCTAAAATGCTAGGAGATTGTCTGTCTGCC-BHQ-3', which were described previously.²⁶ The *fliD* qPCR was done in 25 μ L using FastStart Probe Master mix (Roche, Indianapolis, IN) with 1 μ M each primer, a 250-nM probe, and 5 μ L sample (10% of the total sample).²⁷ The samples, including a positive sample and water controls, were analyzed in a 96-well format with the following parameters: 1 cycle at 95°C for 10 minutes and 40 cycles at 95°C for 10 seconds and 60°C for 1 minute followed by recording using a real-time detection system (Bio-Rad Laboratories, Hercules, CA). Each 96-well plate was analyzed with a 10-fold dilution standard curve of genomic DNA of *B. burgdorferi* B31 (1,200 - 10 pg copies), which also served as a positive control; in addition, a water/no DNA control was performed on each plate. Spirochete numbers in each sample were calculated using Opticon Monitor 3 software, and only those samples having greater than 12 spirochetes (the smallest detectable positive control) were considered positive for *B. burgdorferi* s.s.

DNA extracted from tick samples were then analyzed for the presence of *A. phagocytophilum* as previously described.²⁸ The primer set for *A. phagocytophilum* targeted the major outer surface protein (p44) gene *msp2* as previously described.^{28,29} Amplicons from the tick and mouse DNA were then purified with the QIAquick PCR purification kit (Qiagen, Valencia, CA) and sequenced in both directions using BigDye XTerminator Purification sequencing kits (Applied Biosystems, Carlsbad, CA) with the same primers used for PCR amplification to confirm that positive PCR samples contained *A. phagocytophilum*.

DNA was extracted from small-mammal blood samples (50 μ L whole blood/animal) using the Qiagen DNeasy tissue kit (Valencia, CA) according to the manufacturer's protocol with a final elution volume of 200 μ L. A qPCR assay was used to amplify the mouse actin gene with the Mouse ACTB (actin- β) Endogenous Control (FAM Dye/MGB Probe, Non-Primer Limited; Applied Biosystems, Carlsbad, CA) to verify the presence of murine DNA. qPCR was done in TaqMan Universal PCR Master mix (Applied Biosystems, Carlsbad, CA), with 2.5 μ L primer and probe mix and 2.5 μ L sample (of the 200- μ L DNA purification). Samples were analyzed for mouse actin in a 96-well format with the following parameters: 1 cycle at 95°C for 10 minutes, 45 cycles at 95°C for 10 seconds and 60°C for 1 minute, and 1 cycle at 10°C for 10 seconds using a Chromo4 real-time detection system (Bio-Rad Laboratories, Hercules, CA). The presence of *A. phagocytophilum* was then detected by the PCR target in the p44 gene.²⁸ Sequence analysis of positive amplicons was then determined as described for ticks above.

Analysis of small-mammal blood for doxycycline hyclate.

HPLC was performed to determine plasma pharmacokinetic levels for small mammals as previously described.^{14,15} Approximately 300 μ L whole blood were collected in microtainers containing EDTA and centrifuged, and the plasma was run over a Beckman System Gold high-pressure liquid chromatograph (Beckman Coulter, Fullerton, CA); associated 32-Karat version 5.0 software was used combined with a C_{18} column (100 $\times$ 4.6-mm inside diameter; Alltech, Deerfield, IL).

Testing *B. burgdorferi* field isolates for doxycycline resistance. To determine if any isolates of *B. burgdorferi* from doxycycline bait treatment areas had developed resistance to doxycycline during the course of bait deployment, isolates were subjected to a minimum inhibitory concentration (MIC) and minimum borreliacidal concentration (MBC) assay as previously described.³⁰ Briefly, MBC was determined to be the lowest concentration of doxycycline in which no live spirochetes were observed by microscopy or recovered by incubation in BSK for 21 days, and MIC was determined to be the lowest concentration of cells after incubation that did not exceed the initial number of cells. Two isolates from each treatment area were subjected to testing and compared with a B31 laboratory strain (passage 6; isolated from Shelter Island, NY) as a control. *B. burgdorferi* strains used for control untreated areas were NJ-134 and NJ-141, pre-bait deployment areas NJ-103 and NJ-104, and post-bait deployment areas NJ-129 and NJ-130. Isolates were grown to log phase in BSK-H medium²⁰; 100 μ L *B. burgdorferi* culture at 3×10^5 cells/mL in log phase were placed into a 96-well cell culture plate. Doxycycline hyclate (Sigma-Aldrich, St. Louis, MO) at concentrations from 0.25 to 10 μ g/mL was added to the wells in duplicate in parallel with a 0 μ g/mL control. Plates were allowed to incubate at 34°C for 72 hours; 10 μ L culture from each well were examined by dark-field microscopy for live spirochetes. All isolates from doxycycline concentrations ranging from 0 to 0.75 μ g/mL were placed into fresh BSK-H medium for 21 days to confirm borreliacidal activity. The experiment was repeated three times.

Statistical analyses. The modified Abbott's formula³¹ was used for primary comparisons between untreated control and treatment areas, including percent reduction of *B. burgdorferi* and *A. phagocytophilum* infection rates in small mammals, number of ticks removed from hosts, and questing nymphal and adult ticks. Percent control was calculated as follows: $100 - (\text{treatment/untreated control} \times 100)$. χ^2 tests were used to determine significance (at $P < 0.05$).

RESULTS

Deployment and maintenance of bait stations. After the first week of deployment in May of 2007, 34 of 50 (68%) bait stations showed some consumption of doxycycline bait. By the third week and lasting through 15 weeks, all baits were consumed from 42 (84%) to 50 (100%) bait stations. Mid-week inspections conducted at 4 and 12 weeks showed that, in high-use bait stations, baits were totally consumed after only 4 days of deployment (data not shown). With the appearance of a substantial oak mast at week 16, bait consumption steadily declined (Figure 1).

In 2007, we began to observe squirrel damage to bait stations within 2 weeks of deployment. We typically observed

TABLE 1
Impact of doxycycline hyclate-laden bait on *B. burgdorferi* and *A. phagocytophilum* infection in small mammals

Month	Doxycycline blood level (µg/mL)*		<i>B. burgdorferi</i> infection rate (number positive/total number)		<i>A. phagocytophilum</i> infection rate (number positive/total number)	
	Doxy	Control	Doxy	Control	Doxy	Control
2007						
May	NA*	NA†	8/26 (30%)	9/22 (41%)	17/26 (65%)	14/22 (64%)
June	1.17	0.0	0/40 (0.0%)	19/31 (61%)	3/40 (7.5%)	6/31 (19%)
August	1.42	0.028‡	0/22 (0.0%)	11/27 (41%)	2/22 (9.1%)	7/27 (26%)
2008						
May	NA*	NA†	1/14 (7.1%)	12/33 (36%)	7/14 (50%)	15/33 (46%)
June	1.12	0.027§	0/34 (0.0%)	25/42 (60%)	5/34 (15%)	8/42 (19%)
August	1.29	0.025¶	0/16 (0.0%)	15/34 (44%)	0/16 (0.0%)	8/34 (24%)

* MIC for doxycycline blood levels is ~0.5 µg/mL.¹⁵

† May of 2007 and 2008 were pre-treatment trapping events, and no doxycycline bait was deployed during these periods; therefore, blood was not tested by HPLC. NA = not applicable.

‡ Although there was no doxycycline bait deployed in the untreated control areas, three eastern chipmunks and one eastern gray squirrel had detectable doxy blood levels as determined by HPLC. These animals most likely entered from one of two control areas located ≥ 200 m from the treatment area.

§ A single eastern chipmunk had a detectable blood level of 1.12 µg/mL for doxycycline.

¶ A single eastern chipmunk had a detectable blood level of 0.87 µg/mL for doxycycline.

that the entrance ports of many bait stations were enlarged, presumably by squirrels attempting to access baits. Because the functional integrity of these bait stations was not compromised, they remained in service. However, by the end of the study, squirrel depredation resulted in complete loss and replacement of 57 bait stations (mean = 3.0 ± 3.77 bait stations lost/week, range = 0–7 bait stations/week).

Small-mammal ticks burdens. A total of 3,960 trap nights resulted in 365 small-mammal captures (203 captures from the untreated control area and 162 captures from the treatment area). Eastern chipmunks (*Tamias striatus*) comprised 43.3% of captures, white-footed mice (*Peromyscus leucopus*) were 40.6% of captures, eastern gray squirrels (*S. carolinensis*) were 10.4% of captures, and short-tailed shrews (*Blarina brevicauda*) were 5.7% of captures. A total of 656 ticks were collected from mammal hosts (446 ticks from the two untreated areas and 210 ticks from the treatment area); 91% of the ticks were larval or nymphal *I. scapularis*, and the remaining 9% were immature *Dermacentor variabilis*. Two *Amblyomma americanum* larvae were collected from a squirrel trapped on the untreated control area. Over 2 years, we collected 365 ticks from 157 *T. striatus* (2.3 ticks/chipmunk, 56.3% infestation rate), 225 ticks from 148 *P. leucopus* (1.5 ticks/mouse, 48% infestation rate), 69 ticks from 38 *S. carolinensis* (1.8 ticks/squirrel, 47.4% infestation rate), and 7 ticks from 21 *B. brevicauda* (0.3 ticks/shrew, 28.6% infestation rate).

Infection rates in small mammals. Pre-treatment (May of 2007) *B. burgdorferi* infection rates in small mammals were 41% and 30% for the control and treated areas, respectively (Table 1). After doxycycline bait deployment, not a single infected animal was collected from the treatment area (0% infection rate, $N = 110$; 100% reduction), whereas average infection rates in the control areas were 61% and 41% in June and August, respectively.

Pre-treatment *A. phagocytophilum* infection rates in small mammals from the untreated control and treatment areas were 64% and 65%, respectively (Table 1). Average infection rates in animals recovered from the treatment area in 2007 and 2008 declined to 13.5% (34.7% reduction; $P = 0.04$). The combined percentage of animals coinfecting with both *B. burgdorferi* and *A. phagocytophilum* from both the treated and untreated areas was 13.4%.

***B. burgdorferi* infection in ticks.** Average infection rates in ticks removed from small mammals in the untreated areas throughout the duration of the study was 54.5%. Average pre-

treatment (May of 2007) infection rates in ticks removed from small mammals in the treatment area was 47.7%, and this percentage decreased significantly after bait deployment to 12.3% ($P = 0.03$, 74.2% overall reduction; data not shown).

Initial (May of 2007) average *B. burgdorferi* infection rate in questing nymphal ticks from the untreated areas was 33.1%, which declined slightly to an average of 23.5% in 2008 and 2009 (Table 2). In the treated area, 36.7% of questing nymphs were infected during the pre-treatment period, whereas infection rates declined significantly to 1.9% and 1.5% during 2008 and 2009, respectively, after bait deployment (92.8% control; $P < 0.01$) (Table 2).

The pre-intervention *B. burgdorferi* infection rate in questing adult *I. scapularis* from the untreated areas (65.9%) was significantly greater than the rate observed in the treatment area (30.6%, $P = 0.03$). This finding is most likely attributable to antibiotic baits being available to small mammals for 19 weeks before the adult tick season. Nymphal ticks that fed on treated mice may have been cleared of spirochetal infection, or they may have fed on mice that had been cleared of infection and therefore, were uninfected during the molt to the adult stage. The infection rate in questing adult ticks after 1 year of bait deployment was decreased by 26.9% (Table 3).

***A. phagocytophilum* infection in questing ticks.** Pre-intervention infection rate in questing nymphal ticks collected in the treatment area was 16.3%. After 2 years of bait deployment (38 weeks total), infection rates declined > 10-fold to 1.5% (72% reduction) (Table 2). Infection rates in adult ticks declined from 20.4% in May of 2007 to 7.7%

TABLE 2
Impact of doxycycline hyclate-laden bait on *B. burgdorferi* and *A. phagocytophilum* infection in questing nymphal *I. scapularis* ticks

Date of collection	<i>B. burgdorferi</i> infection rate (number positive/total number)*	<i>A. phagocytophilum</i> infection rate (number positive/total number)†
Doxy bait area		
2007*	18/49 (36.7%)	8/49 (16.3%)
2008	1/52 (1.9%)	4/52 (8.2%)
2009	1/66 (1.5%)	1/66 (1.5%)
Untreated area		
2007	17/58 (33.1%)	13/58 (22.4%)
2008	15/56 (26.8%)	11/56 (19.6%)
2009	8/42 (19.1%)	7/42 (16.7%)

* Percent infection of nymphal *I. scapularis* ticks for *B. burgdorferi* and *A. phagocytophilum* was determined by PCR.

† Pre-intervention.

TABLE 3

Impact of doxycycline hyclate-laden bait on *B. burgdorferi* and *A. phagocytophilum* infection in questing adult *I. scapularis* ticks

Date of collection	<i>B. burgdorferi</i> infection rate (number positive/total number)*	<i>A. phagocytophilum</i> infection rate (number positive/total number)*
Doxy bait area		
2007	15/49 (30.6%)	10/49 (20.4%)
2008	11/52 (21.2%)	4/52 (7.7%)
2009	6/50 (12.0%)	NA†
Untreated area		
2007	31/47 (65.9%)	17/37 (36.1%)
2008	49/93 (52.7%)	13/59 (22.03%)
2009	29/55 (52.7%)	NA

* Percent infection of adult *I. scapularis* ticks for *B. burgdorferi* and *A. phagocytophilum* was determined by PCR.

† Study was terminated after nymphal collection in June of 2009. NA = not applicable.

in 2008 (1 year of bait deployment, 19 weeks) (Table 3). This finding resulted in a significant decrease of 59.7% ($P = 0.04$) (Table 3). Adult ticks collected in the fall of 2009 were not tested for infection. We were unable to test adult ticks for *A. phagocytophilum* in November of 2009, because DNA was not available from these ticks.

Doxycycline resistance to *B. burgdorferi* in small-mammal isolates. A total of six field isolates were tested for *B. burgdorferi* resistance to doxycycline hyclate. Samples tested included two *B. burgdorferi*-positive cultures from the untreated control areas and four cultures from the treated area. Two of four treatment area isolates came from ear biopsy-positive mammals trapped in May of 2007 during the initial pre-treatment trapping event. During the months of bait deployment, there were no culture-positive small mammals trapped in the treatment area ($N = 110$). The remaining two *B. burgdorferi*-positive isolates tested were obtained during the month of May both in 2008 and 2009. All isolates tested, including *B. burgdorferi* B31 control, were completely susceptible to doxycycline hyclate at the lowest concentration tested (0.25 µg/mL), and attempts to grow spirochetes from the lowest concentrations tested were unsuccessful, indicating there was no development of resistance to doxycycline in these field trials.

DISCUSSION

This field trial showed that targeted delivery of bait containing doxycycline hyclate reduced *B. burgdorferi* and *A. phagocytophilum* infection rates in small mammals and subsequently, questing *I. scapularis* populations. Consumption data showed that the 500 mg/kg doxycycline hyclate-impregnated baits were palatable and that target small mammals rapidly acclimated to the bait stations. Within several weeks of deployment, all bait was removed from > 95% of bait stations, and in most cases, this removal occurred within 4 days of deployment. Presumably, the bait was consumed within the bait station or removed and cached by small mammals, particularly squirrels and eastern chipmunks, for later consumption. During the first week of September of 2007 (week 16 post-bait box deployment), we experienced substantial oak masting, and bait consumption steadily declined from a high of 100% in week 6 to only 4% by week 19. Small mammals often switch foraging preferences as the amount and type of food available changes during a given year.^{32,33} In addition, the works by Jones and others³⁴ and Ostfeld and others³⁵ showed that interannual variation in the abundance of preferred foods (including acorns)

may directly influence fluctuations in the density of tick hosts and consequently, the ecological risk of exposure to Lyme disease. Therefore, the efficacy of host-targeted tick control technologies that rely on a bait source, such as the one described here, may be less effective during times when there is an abundance of alternative food sources.

Previous laboratory trials using the same 500-mg/kg formulation of doxycycline hyclate-laden bait showed 100% prophylaxis and clearing of acute established *B. burgdorferi* infection in C3H/HeJ mice.¹⁴ The field trial described here suggests that consumption of this bait by wild-trapped small mammals had a significant impact on the natural enzootic cycles of both *B. burgdorferi* and *A. phagocytophilum*, impacting both *I. scapularis* and its reservoir hosts. Ear-biopsy cultures from small mammals showed that the enzootic cycle for both infections in this area of central New Jersey is very intense. Average *B. burgdorferi* and *A. phagocytophilum* infection rates in small mammals in the untreated area during this 2-year study were 47.2% and 33%, respectively. Placement of antibiotic bait stations early in the spring when transmission usually occurs cleared many spirochete-infected small mammals that were being fed on by infected nymphal ticks. After bait box deployment, infection rates in small mammals in the treated areas were reduced by 86.9% and 74% for *B. burgdorferi* and *A. phagocytophilum*, respectively. Our recapture data also supports the ability of doxycycline bait to prophylactically protect and cure small mammals and ultimately, impact the Lyme disease enzootic cycle. Of 365 total captures, there were a total of 46 recaptures (12.6% recapture rate). In the treatment area, 22 of 23 (95.7%) small mammals were either cured (4/23; 17%) or prophylactically protected against (18/23; 78.3%) *B. burgdorferi* infection compared with 15 of 23 (65.2%) that became infected during the trial or were previously infected and remained so in the untreated control areas. This rate equates to a 91.7% reduction in the number of infected small-mammal reservoirs in the doxycycline bait-treated areas. Similarly, 12 of 23 (52.2%) small mammals were cured, and 10 of 23 (43.5%) were prophylactically protected against *A. phagocytophilum* infection (22/23 total; 95.7%), where 11 of 23 (47.8%) small mammals from the untreated control area either acquired infection or were already infected and remained so during the trial.

Multiple microorganisms have been shown to cocirculate in *I. scapularis* ticks collected previously in northern New Jersey, including ticks dually infected with both *B. burgdorferi* and *A. phagocytophilum*.³⁶ Small-mammal reservoirs are routinely found to be infected with multiple tick-borne pathogens as well.³⁷ We trapped 33 animals that were coinfecting with *B. burgdorferi* and *A. phagocytophilum* (28 animals from the untreated area and 5 animals from the treated area captured before bait box deployment). All five of the latter animals were subsequently recaptured during periods of bait deployment, and all were found to be negative for both *B. burgdorferi* and *A. phagocytophilum* infection. A single chipmunk that was found to be negative for both infections during May of 2007 was positive for both when later captured in May of 2008 during a period between bait deployments. In a laboratory study, Zeidner and others¹⁶ showed the ability of a sustained release formulation of doxycycline hyclate (Atridox) to prophylactically protect against simultaneous infection of *B. burgdorferi* and *A. phagocytophilum* in C3H/HeJ mice by tick bite. However, we believe our study to be the first field

trial of a novel technology to show the simultaneous cure of both *B. burgdorferi* and *A. phagocytophilum* in wild populations of small mammalian reservoirs.

Ticks removed from small mammals in the treatment area during the months of bait deployment showed a > 3.8-fold reduction in *B. burgdorferi* infection rates. However, unlike in a previous laboratory study,¹⁴ we were unable to show complete clearing of ticks of *B. burgdorferi* infection. In the laboratory, mice were provided the doxycycline bait during the duration that ticks fed (~96 hours to repletion). In contrast, we were unable to determine how many times individual animals visited bait boxes or how much bait they consumed. HPLC testing showed that a high percentage (77.8%) of mammal hosts from the treatment area had MIC blood levels of doxycycline hyclate in plasma. Because ticks that were removed from hosts captured in the treated area and analyzed by PCR were at various stages of feeding, some were nearly engorged, and some were newly attached and relatively flat. Ticks in early stages of feeding may not have taken in sufficient levels of antibiotic to clear them of spirochetal infection, or their hosts may not have had sufficient pharmacokinetic levels to clear infection. Future studies should include holding animals over water until all ticks are fed to repletion before PCR analysis to test for complete clearing. We tested *B. burgdorferi* strains collected from the test area to be certain antibiotic resistance was not selected for during our pilot study. No evidence of antibiotic resistance was found. However, the use of antibiotic-laden baits for the prevention of Lyme disease in the field would require close monitoring to be certain that antibiotic resistance did not arise.

The number of Lyme disease cases reported to the Centers for Disease Control and Prevention (CDC) continues to exceed 25,000/year. There is a continued need to develop and test novel approaches to tick control that can be effective either alone or as part of an integrated tick management (ITM) approach on residential properties and public lands that minimize the use of synthetic acaricides and emphasize the least-toxic methods.

The use of host-targeted bait boxes containing the acaricide fipronil has been shown to be effective at reducing tick burdens on mice and chipmunks, numbers of questing ticks, and infection rates in rodent reservoirs. In a 3-year study on a coastal island of Connecticut, bait boxes reduced immature *I. scapularis* burdens on mice by 76%, questing nymphal populations by > 50%, and the number of infected mice by > 57%.¹¹ In a New Jersey field trial, simultaneous deployment of US Department of Agriculture 4-Poster deer feeders and MaxForce TMS bait boxes was used to passively treat deer, white-footed mice, and eastern chipmunks, and it resulted in a 94.3% reduction of host-seeking nymphal ticks after 2 years.¹² In the present study, the use of doxycycline-treated bait significantly reduced the percentage of rodent reservoirs infected with *B. burgdorferi* by 86.9% compared with > 50% for the fipronil-treated bait boxes. However, although the use of antibiotic bait was extremely effective at reducing infection rates in small mammals and questing tick populations (> 92%), this method alone will not reduce questing tick populations and would provide the greatest benefit if used as part of an ITM approach.

A novel doxycycline hyclate-impregnated bait formulation was shown to be highly efficacious in providing prophylaxis against tick-transmitted *B. burgdorferi* and *A. phagocytophilum*, curing infected small mammals, and reducing infection

rates in questing ticks. We recognize that distributing doxycycline-impregnated bait for controlling tick-borne diseases may be controversial given the fact that doxycycline is routinely prescribed for treating these infections in patients. Although the results reported here serve as proof of concept, studies are underway to evaluate alternative antibiotics with similar anti-spirochetal activity that do not serve as front-line drugs for the treatment of Lyme disease, Rocky Mountain spotted fever, or human granulocytic anaplasmosis.

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Disclaimer: The findings and conclusions of this study are by the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention. Mention of a method or product does not constitute an endorsement. Animals were handled according to approved protocols on file with the Centers for Disease Control and Prevention Division of Vector-Borne Diseases Animal Care and Use Committee Protocol number 08-008.

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