# Effects of Tick-Control Interventions on Tick Abundance, Human Encounters with Ticks, and Incidence of Tickborne Diseases in Residential Neighborhoods, New York, USA

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Tickborne diseases (TBDs) such as Lyme disease result in ≈500,000 diagnoses annually in the United States. Various methods can reduce the abundance of ticks at small spatial scales, but whether these methods lower incidence of TBDs is poorly understood. We conducted a randomized, replicated, fully crossed, placebo-controlled, masked experiment to test whether 2 environmentally safe interventions, the Tick Control System (TCS) and Met52 fungal spray, used separately or together, affected risk for and incidence of TBDs in humans and pets in 24 residential neighborhoods. All participating properties in a neighborhood received the same treatment. TCS was associated with fewer questing ticks and fewer ticks feeding on rodents. The interventions did not result in a significant difference in incidence of human TBDs but did significantly reduce incidence in pets. Our study is consistent with previous evidence suggesting that reducing tick abundance in residential areas might not reduce incidence of TBDs in humans.

Lyme disease is an emerging zoonosis caused by the spirochete bacterium *Borrelia burgdorferi*, which is transmitted between vertebrate hosts, including humans, by ticks in the *Ixodes ricinus* complex. Annual cases of Lyme disease in the United States, as reported to the Centers for Disease Control and Prevention (*1*), have grown from a few hundred in the early 1980s to >30,000 in recent years. A recent study estimated that actual clinician diagnoses of Lyme disease in the past decade exceed 450,000 per year (*2*,*3*). Increasing incidence over the past few decades reflects both upward trends in case numbers within Lyme disease–endemic locations and a dramatic geographic spread from both northeastern and Midwestern foci (*4*–*6*). Beyond the effects of Lyme disease on human health, economic costs of patient care are estimated at ≈$1 billion/year in the United States (*7*).

Preventing exposure to *B. burgdorferi* and other tickborne pathogens can be aided by personal practices such as applying repellents, checking for ticks, and avoiding tick habitats. However, the efficacy of these methods is unclear, and considerable differences in effects have been reported (*8*,*9*). Although specific methods of property and wildlife management (e.g., deer hunting) are advocated by some agencies (*10*), knowledge of the effectiveness of these recommendations in reducing human encounters with ticks and incidence of tickborne diseases (TBDs) is limited (*11*–*13*).

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DOI: https://doi.org/10.3201/eid2805.211146

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Controlling the size of tick populations is generally considered a promising way of reducing human exposure to TBDs. Researchers pursuing these methods have identified chemical and biological agents, including synthetic pyrethroids, organophosphates, and entomopathogenic fungi, that are lethal to ticks (*14*–*19*). Field trials generally show that application of chemical or biologic acaricides can reduce the number of ticks by 50%–90% (*20*–*22*). Combining acaricides with other interventions (e.g., wildlife and landscape management) has also been assessed. However, studies evaluating whether these integrated approaches reduce human exposure to ticks are limited by design constraints, such as the lack of masking of researchers to treatment assignments, lack of appropriate placebo controls, small scale of deployment, unbalanced designs, and low statistical power. Studies also do not generally include data on human health outcomes, particularly incidence of TBDs (*23*,*24*).

A recent study (*23*) rectified many of these deficiencies by applying an acaricide (bifenthrin) to 2,727 residential properties in 3 states; using a masked, placebo-controlled design; and including tick abundance, human encounters with ticks, and cases of TBDs as response variables. Despite showing >60% reduction in tick populations on properties treated with the acaricide versus the placebo control (water), the study (*23*) showed no reduction in either tick encounters or cases of TBDs. One potential reason for this lack of effect is that the treatments did not reduce tick abundance below some putative threshold needed for reduced disease risk. A second possibility is that humans might frequently encounter ticks in locations other than their yards. In both cases, tick control might be more effective at reducing tick exposures when applied throughout a residential neighborhood.

This study, the Tick Project (*25*), was designed to determine whether tick control, when implemented more broadly in residential neighborhoods and by using multiple approaches to tick management, could reduce TBD risk and incidence. We designed a randomized, replicated, fully crossed, placebo-controlled, masked experiment to evaluate whether 2 environmentally safe methods to manage ticks, used separately or together, reduced tick abundance, human and pet encounters with ticks, and human and pet cases of TBDs.

## Methods

We tested the effects of 2 methods of tick control, used separately or together, on tick abundance, tick encounters with humans and pets, and cases of TBDs over 4 years (2017–2020) in 24 neighborhoods in Dutchess County, New York, USA. The first intervention, the Tick Control System (TCS) (Select TCS, Tick Box Technology Corporation, http://www.tickboxtcs.com), consists of baited boxes that attract the small mammal hosts most likely to infect ticks with pathogens. When inside the box, these mammals are brushed with a dose of the acaricide fipronil. The second intervention, Met52 (Novozymes Biologicals, https://biosolutions.novozymes.com), is a fungal spray developed to kill questing ticks. Both interventions have been demonstrated to have extremely low toxicity to humans, pets, and wildlife as applied (*21*); high specificity for ticks (*26*); evidence of efficacy in tick-control as revealed in small-scale studies (*15*,*20*–*22*,*27*); and commercial availability at the time of the study.

The design was fully crossed so that 4 treatments were used: placebo TCS boxes and placebo Met52, placebo TCS boxes and active Met52, active TCS boxes and placebo Met52, and active TCS boxes and active Met52. All participating properties within a neighborhood received the same treatment. We included 6 replicate neighborhoods in each of 4 treatment categories to achieve 80% power to detect an effect size of 60%. Given the intensity of treatments and length of the study, increasing the sample size to achieve greater power was infeasible. Selected neighborhoods had high incidence of Lyme disease and moderate to high density of 1- and 2-family residences. During April 2016–June 2017, residents were recruited by mail, telephone, and in-person visits. Neighborhood treatments were randomly assigned, and study participants and scientific personnel that collected or managed data on response variables were masked to treatment assignments (Appendix).

Beginning in spring 2017, we deployed the 4 treatment combinations on participating properties (Appendix Table 1, https://wwwnc.cdc.gov/EID/article/28/5/21-1146-App1.pdf). We deployed TCS boxes or placebo boxes that contained no acaricide at densities consistent with product labeling during spring and summer, corresponding to the activity peaks for nymphal and larval blacklegged ticks (*28*). We placed boxes ≥10 meters apart in all habitat types that we sampled for ticks and placed them in protected locations, such as along building foundations and under vegetation, that are frequently used by small mammals.

If effective, TCS bait boxes would kill larval (hatchling stage) ticks feeding on small-mammal hosts in summer and fall, leading to fewer nymphs

(second immature stage) the following spring. Met52 would kill questing nymphal ticks in spring. Our tick sampling focused on the abundance of questing nymphal ticks in spring and ticks on small mammals in summer.

Met52, which contains spores of the F52 strain of the entomopathogenic fungus *Metarhizium brunneum*, was prepared according to product label instructions and applied by using truck-mounted high-pressure sprayers. Identical trucks and sprayers were filled with water for the placebo controls. Spraying was conducted twice each year preceding and during the peak of activity of questing nymphal ticks (*28*). For properties that included extensive forested areas, spraying extended 12 meters into the forest.

During the peak activity period for questing nymphal ticks and at least 1 week after spraying, we used 1-m × 1-m white corduroy cloth to flag-sample ticks at 20 randomly chosen participating properties within each neighborhood, sampling 3 habitat types on each property: lawn, forest, and shrub or garden, whenever present. To assess tick burdens on small mammals, we conducted mark-recapture sampling by using Sherman live traps at 10 participating properties in each neighborhood during August and September 2017–2019, corresponding to the activity peak of the larval stage (*28*). We did not conduct sampling in 2020 because of the coronavirus disease pandemic.

In an introductory survey, we asked the primary contact for each household where and how frequently each member of the household spent time outdoors and what approaches to personal tick prevention they used. From spring through late fall each year (Appendix Table 2), we distributed biweekly surveys to each participating household, asking whether any full-time resident, including pets that spent time outdoors, had encountered a tick or had a TBD diagnosed in the previous 2 weeks. We asked participants who reported TBD in humans to consider signing a medical consent form to enable confirmation of the case by their healthcare provider.

We generally evaluated effects of treatments by analyzing data aggregated at the neighborhood level to determine the effects of each treatment alone and in combination (Appendix). For tick encounters and cases of TBDs for humans and pets, we accounted for numbers of participants within neighborhoods. The Institutional Review Board and the Institutional Animal Care and Use Committee of the Cary Institute of Ecosystem Studies (Millbrook, NY, USA) approved protocols involving informed consent by human participants and the live-trapping and handling of small mammals.

## Results

### Characteristics of Neighborhoods and Participants

The average neighborhood was 27.5 (range 12.9–39.2) hectares and contained 118 (range 77–162) properties; average parcel size was (range 0.02–1.8) 0.19 hectares. A mean of 43% (range 18%–63%) of the neighborhood consisted of forested habitat, whereas lawns, shrubs, and gardens together accounted for ≈30% (range 14%–48%).

During the recruitment phase, ≈25% of households in each neighborhood did not respond to repeated attempts at contact, ≈25% declined to participate, and ≈10% were either ineligible (e.g., because they used pesticides) or failed to fully enroll (Appendix Figure 1). By the end of the recruitment phase, an average of 34% (range 24%–44%) of the properties in a given neighborhood were enrolled in the project. Neither the proportion of properties enrolled (Appendix Table 3, Figure 1) nor the habitat composition of the neighborhoods (Appendix Tables 4, 5) varied significantly by treatment group.

When the study began, a mean of 101 (range 62–136) persons and 35 (range 14–58) outdoor pets were enrolled in each neighborhood, for a total of 2,384 human participants and 849 pets. Enrollment numbers did not vary significantly by treatment group (Table 1). On average, participants had a median age of 49 years, and 40% of households had an annual

**Table 1.** Characteristics of participants for the 24 residential neighborhoods together and for the 6 neighborhoods in each of the 4 treatment groups of tick-control interventions, New York, USA*

| Characteristic | Overall | Neither active | Active Met52 | Active bait boxes | Both active |
|---|---|---|---|---|---|
| No. neighborhoods | 24 | 6 | 6 | 6 | 6 |
| Mean no. human participants per neighborhood | 97 (± 19) | 110 (± 13) | 94 (± 26) | 94 (± 13) | 90 (± 18) |
| Mean no. outdoor pets per neighborhood | 30 (± 8) | 26 (± 9) | 33 (± 9) | 29 (± 5) | 31 (± 10) |
| Average median age of human participants, y | 49 (± 5) | 48 (± 4) | 51 (± 3) | 48 (± 6) | 49 (± 6) |
| Per capita no. preventive behaviors | 1.27 (± 0.27) | 1.20 (± 0.35) | 1.37 (± 0.27) | 1.27 (± 0.24) | 1.27 (± 0.24) |
| Self-reported cases of diagnosed TBDs per capita before study onset, 2011–2016 | 0.07 (± 0.03) | 0.05 (± 0.02) | 0.07 (± 0.03) | 0.07 (± 0.02) | 0.07 (± 0.05) |

*Data on age, previous cases of TBDs, and preventive behaviors were self-reported on the introductory survey administered during 2016–2017. Data on the number of participants and pets who spent time outside were averaged over the length of the study. Values in parentheses represent the standard error of the mean. TBDs, tickborne diseases.

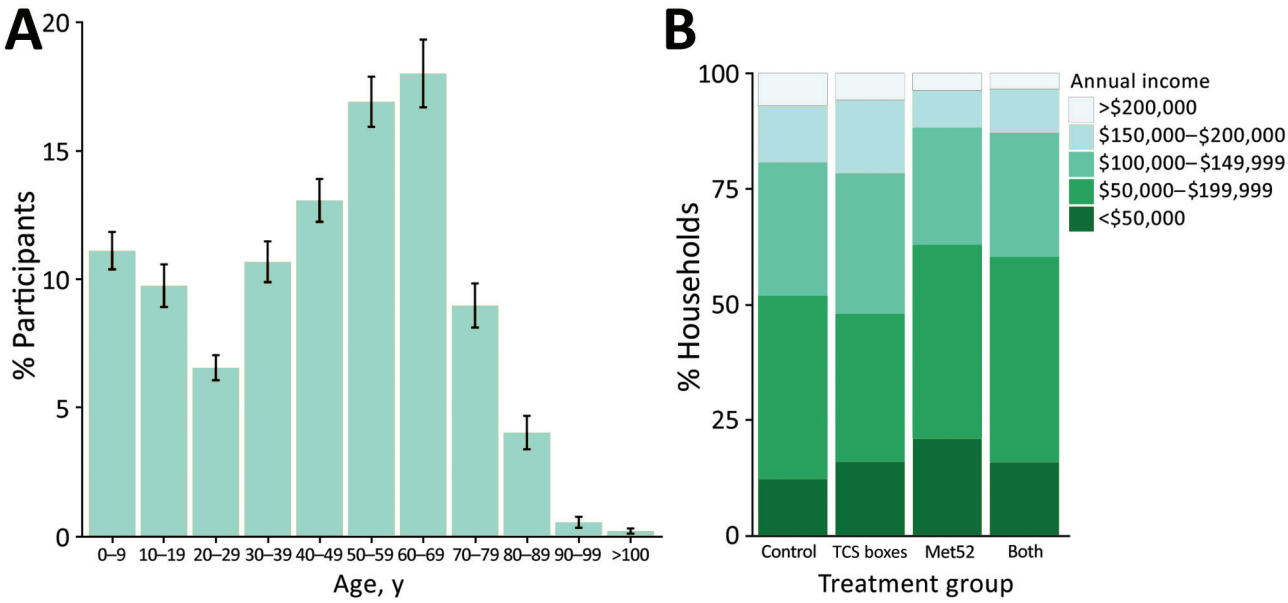


**Figure 1.** Characteristics of participants in study of tick-control interventions in residential neighborhoods, New York, USA. A) Mean percentage of participants in each age category at the time of enrollment, averaged for 24 neighborhoods. Error bars represent SEM. B) Mean percentage of households in each category of annual household income, averaged for the 6 neighborhoods in each treatment group. TCS, Tick Control System.

household income of $50,000–100,000 (Figure 1). Participants reported that when they spent time outside, most of their time was spent on their own properties or away from their neighborhoods (Appendix Figure 4). Participants reported regularly practicing just over 1 preventive behavior (e.g., tick checks) to protect themselves from ticks and TBDs (mean 1.2 ± 0.3 SEM; Table 1).

## Tick Abundance

### Questing Nymphal Ticks

Per sampling interval, more of the 4,040 questing nymphal ticks collected in the study were found in forested areas of properties than on lawns or in shrubs or gardens (Figure 2, panel A). At the neighborhood level of analysis, the presence of active TCS boxes was associated with a 53% reduction in the number of questing nymphal ticks in forest habitats compared with placebo controls, a statistically significant difference (Figure 2, panel A; Appendix Table 6). Despite an apparent reduction in tick abundance (compared with placebo controls) associated with Met52 treatment in forest habitats (Figure 2, panel A), this effect was not statistically significant, nor was there a significant effect of the 2 treatments used together (a significant interaction) (Appendix Table 6). Shrub and garden habitats showed a similar pattern; 40% fewer questing nymphal ticks were detected on properties with active TCS boxes than those with placebo controls (Figure 2, panel A; Appendix Table 7). This effect was statistically significant, but no significant effect of either active Met52 or the 2 treatments together was seen (Figure 2, panel A; Appendix Table 7). In lawn habitats at the neighborhood level of analysis, no statistically significant effect of either of the treatments used alone or together was seen (Figure 2, panel A; Appendix Table 8).

At the property level, ticks were detected in forested habitats on 75% of properties that received no active treatments but on only 45% of properties treated with active TCS boxes (Figure 2, panel B). A similar and statistically significant pattern was observed for the other 2 habitat types (Figure 2, panel B; Appendix Tables 9–11). There was no significant effect of active Met52 on the probability of detecting ticks in any of the 3 habitats, nor was there an effect of the treatments used together.

### Larval and Nymphal Tick Burdens on Small Mammals

Averaged across all years and all treatments, white-footed mice had mean (± SEM) tick burdens of 3.7 ± 0.4 ticks/animal and chipmunks had 0.7 ± 0.1 ticks/animal (Figure 3). The presence of active TCS boxes was associated with a reduction in the mean number of ticks per white-footed mouse by about half (Figure 3, panel A; Appendix Table 12). There was no significant effect of either active Met52 or the treatments together on the average tick burden on mice (Appendix

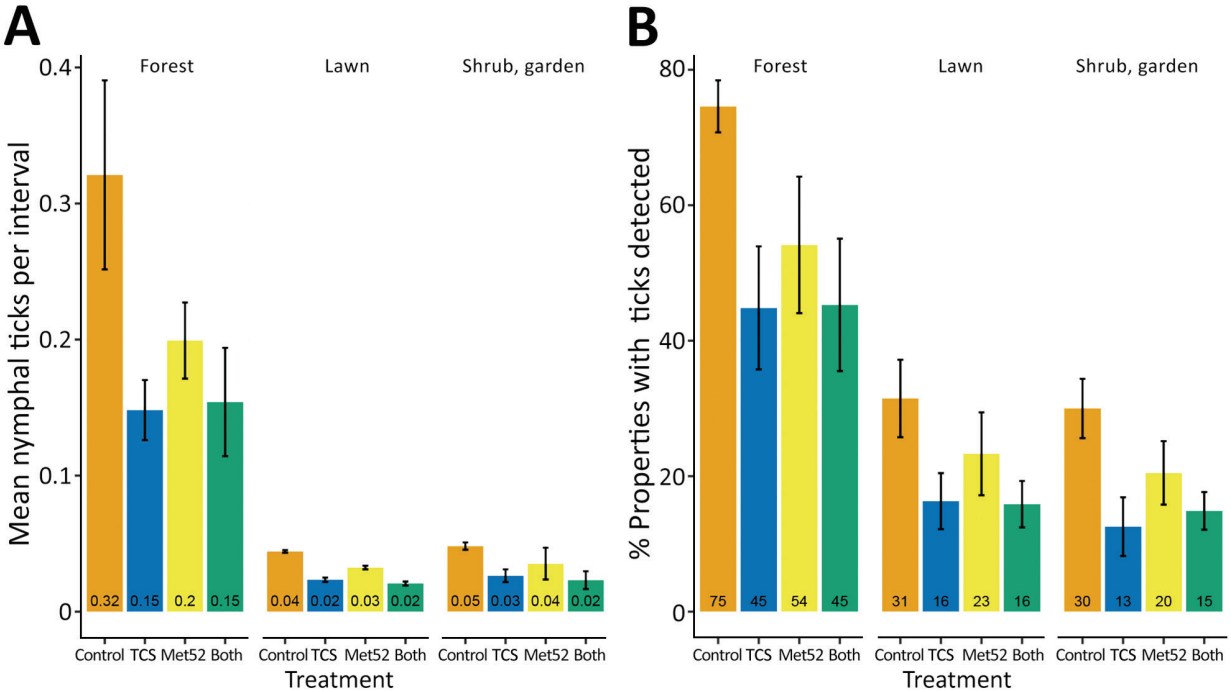


**Figure 2.** Detection of questing nymphal ticks during study of tick-control interventions in residential neighborhoods, New York, USA. A) Mean number of questing nymphal ticks per flagging interval (Appendix, https://wwwnc.cdc.gov/EID/article/28/5/21-1146-App1.pdf). B) Mean percentage of properties with questing nymphal ticks detected for each treatment group and in each habitat type (forest, lawn, shrub or garden). Totals are averaged over 3 years for each neighborhood. Data include ticks from the nymphal sampling period in May–July. Error bars represent SEM. TCS, Tick Control System.

Table 12). Neither treatment had a significant effect on the probability of tick presence on chipmunks or on nonzero tick burdens on chipmunks (Figure 3; Appendix Table 13).

### Case and Encounter Data for Humans

We received 1,664 reports of encounters between ticks and human participants. The cumulative number of reported human encounters with ticks was ≈20% lower in neighborhoods treated with both active TCS boxes and active Met52, but this difference was not statistically significant (Figure 4, panel A), nor was there a significant effect of either of the active treatments alone (Appendix Table 14).

We received a total of 130 reports of TBD diagnoses in humans during 2017–2020. The active treatments, either alone or in combination, demonstrated no effect on the number of self-reported human cases of TBDs (Table 2; Figure 4, panel C; Appendix Table 15). We received permission to pursue confirmation for 84 (65%) of these cases and received 52 responses from healthcare providers. Of these, 35 (67%) confirmed

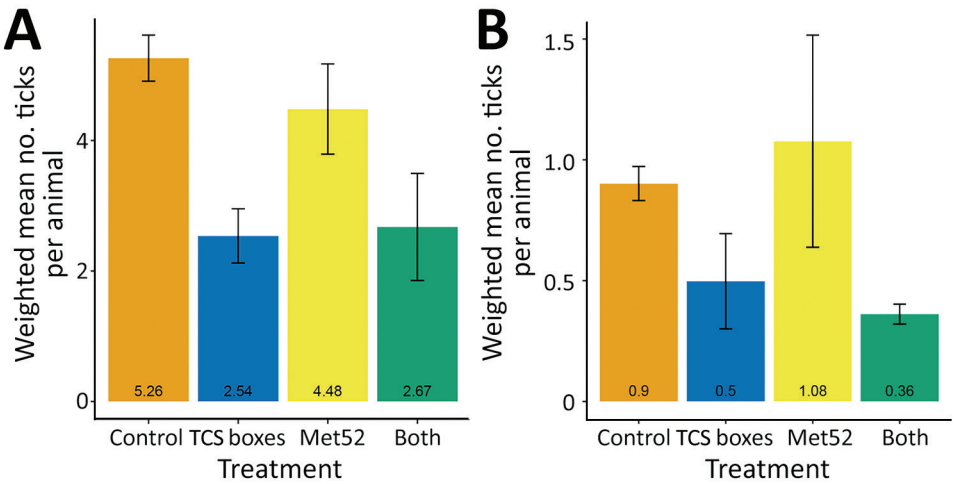


**Figure 3.** Weighted mean number of ticks on white-footed mice (A) and chipmunks (B) as a function of tick-control treatment, New York, USA, 2017–2019. Means represent the average of the 6 neighborhoods in each treatment group, whereas error bars represent SEs. Note that the scale of the y-axes differs. TCS, Tick Control System.

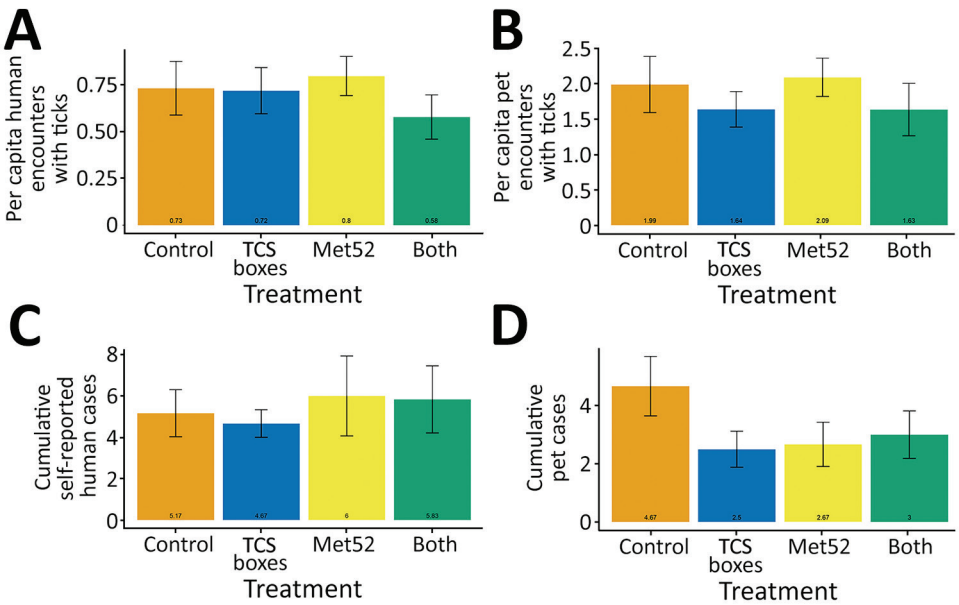


**Figure 4.** Mean per capita human and pet encounters with ticks and cumulative numbers of cases per neighborhood of tick-borne diseases for humans and pets in study of tick-control interventions, New York, USA. A) Human encounters; B) pet encounters; C) self-reported human cases; D) pet cases. Data represent the mean of the cumulative value (± SEM) over the 4 years of treatments (2017–2020), averaged across neighborhoods in a treatment group. Note that the scale of the y-axes differs. TCS, Tick Control System.

diagnoses of a TBD. There was no significant effect of the active treatments, either alone or in combination, on the number of human cases of TBDs confirmed by healthcare providers (Table 2; Appendix Table 16).

### Case and Encounter Data for Pets

We received 1,307 reports of tick encounters for outdoor pets during 2017–2020. The cumulative number of reported pet encounters with ticks was ≈20% lower in neighborhoods with active TCS boxes, but this difference was not statistically significant, nor was there a significant effect of active Met52 treatments (Figure 4, panel B; Appendix Table 17). We received 77 reports of TBD diagnoses in pets during 2017–2020, as reported by owners. The incidence of owner-reported cases of TBDs in pets was lower by about half in neighborhoods with active TCS boxes or active Met52, and these differences were statistically significant (Table 2; Figure 4, panel D; Appendix Table 18).

### Effectiveness of Masking Procedures

Of 874 households participating in December 2020, a total of 507 primary contacts (58%) completed the final survey; 438 (86%) of those contacts said they did not know their neighborhood's treatment assignment. Of the 65 who thought they knew their neighborhood's treatment assignment, their guesses were incorrect (54%) more frequently than they were correct (46%) (Appendix).

## Discussion

We conducted a large-scale, randomized, masked, placebo-controlled study of the effects of 2 methods of tick control in residential neighborhoods. The central goal was to evaluate whether community-level control of ticks could reduce the threat of TBDs to public health. We documented significant reductions in tick abundance within certain treatment groups, most consistently within forest and garden habitats. These effects were not associated with significant reductions in human exposure to ticks or TBDs. However, TBD incidence in outdoor pets was significantly lower in neighborhoods that received the interventions.

Deploying active TCS boxes in neighborhoods was associated with fewer questing nymphal ticks by >50% and fewer ticks on rodents by ≈50% compared with placebo controls. Active Met52 spray showed no effect on the abundance of either questing or attached ticks compared with placebo controls. Not surprisingly, using those 2 methods of tick control together did not show multiplicative effects, as indicated by the lack of statistically significant interactions between the interventions.

The protocols for TCS and Met52 used in this study complied with product labels. The low efficacy of Met52 may have arisen from degradation and low residual effects of the acaricide after applications (*29*). Other studies using TCS boxes or Met52 are not directly comparable to ours because they used multiple tick-control methods with unbalanced designs or lacked placebo controls (*20*,*21*,*30*), which are necessary to account for the presence of the food and shelter TCS boxes provide and to ensure that personnel collecting data are unaware of treatment assignments. Also, previous studies have

tended to restrict TCS box placement or Met52 application to habitat edges, whereas we treated more broadly across habitat types. For example, a recent study placed TCS boxes in a single line along forest–lawn ecotones and found no effect (*31*). Keeping these important differences in mind, the reductions we observed in questing ticks and ticks on rodents in the neighborhoods with active TCS boxes and active Met52 were similar in magnitude to some previous studies using these tick-control methods (*22*) but differed from others (*15*,*20*,*31*,*32*).

Human encounters with ticks have been demonstrated to be a proxy for cases of TBD (*33*). We received 20% fewer cumulative reports of encounters between human participants and ticks, and between outdoor pets and ticks, in neighborhoods treated with both active TCS boxes and active Met52 than for placebo controls. However, this difference was not statistically significant, which might have been caused by stochastic variation among neighborhoods associated with relatively low numbers of cases.

The weak effects of tick reduction on tick encounters and reported cases of TBDs in humans could have arisen from one or more of the following reasons. First, despite persistent, energetic efforts throughout the first year of the study to recruit as many households as possible within neighborhoods, we enrolled 24%–44% of the households in each neighborhood (Appendix Figure 1). Although dozens of individual properties were treated per neighborhood, these treated areas might have been too sparse to provide added benefits over the treatment of individual properties. If more households in each neighborhood had participated, we might have observed greater reductions in tick numbers and an associated reduction in incidence of TBDs. However, increasing participation substantially in future interventions targeted at neighborhoods might not be feasible (Appendix Figure 1). General enthusiasm among residents was high, and the retention and response rates suggest high motivation among those who did participate.

Second, a total of 130 cases of TBDs were reported for all 24 neighborhoods cumulatively over the 4 years of treatments in this study, for a mean of only 5.5 cases per neighborhood. Such a low number of cases might have curtailed our ability to detect effects of the interventions. However, despite only 77 reported cases of TBDs in outdoor pets, or 3.7 cases per neighborhood on average, we detected a significant reduction in neighborhoods with active interventions compared with placebo controls. The absence of effects of treatment on incidence of self-reported and physician-confirmed cases of TBD in humans cannot be attributed solely to a limited number of cases.

A third possibility, related to the second, is that residents of our focal county frequently take actions to prevent exposure to tick bites and tickborne pathogens, which might have limited the effects observed from the interventions. Our study population within Dutchess County, New York, began experiencing high exposure to Lyme disease and other TBDs in the early 1990s, and many residents

**Table 2.** Cumulative diagnosed cases of tickborne diseases, averaged across the 6 residential neighborhoods in each treatment group of tick-control interventions, New York, USA*

| Cases and treatment groups | Per capita cases (SE) | Cases/neighborhood (SE) | p value |
|---|---|---|---|
| Cases of diagnosed tickborne diseases in humans reported by participants, n = 130 | | | |
| Control | 0.05 (0.01) | 5.17 (2.11) | |
| Active TCS boxes | 0.05 (0.01) | 4.67 (1.91) | NS |
| Active Met52 | 0.06 (0.02) | 6.00 (2.45) | NS |
| Active TCS boxes and active Met52 | 0.06 (0.01) | 5.83 (2.38) | NS |
| Cases of diagnosed tickborne diseases in humans confirmed by healthcare providers, n = 35† | | | |
| Control | 0.009 (0.00) | 1.00 (0.41) | |
| Active TCS boxes | 0.012 (0.00) | 1.17 (0.48) | NS |
| Active Met52 | 0.019 (0.01) | 2.17 (0.88) | NS |
| Active TCS boxes and active Met52 | 0.016 (0.01) | 1.50 (0.61) | NS |
| Cases of diagnosed tick-borne diseases in outdoor pets reported by participants, n = 77 | | | |
| Control | 0.17 (0.03) | 4.67 (1.91) | |
| Active TCS boxes | 0.08 (0.02) | 2.50 (1.02) | ‡ |
| Active Met52 | 0.08 (0.03) | 2.67 (1.09) | ‡ |
| Active TCS boxes and active Met52 | 0.11 (0.04) | 3.00 (1.22) | NS |

*For detailed statistical results, see Appendix Tables 16, 18, and 19 (https://wwwnc.cdc.gov/EID/article/28/5/21-1146-App1.pdf). Data represent the mean of the cumulative value (±SEM) over the 4 years of treatments, averaged across neighborhoods in a treatment group. NS, not significant.
†Cases in humans confirmed by healthcare providers were less common than cases reported by participants because some participants did not grant permission to the investigators to pursue confirmation from healthcare providers, some healthcare providers did not respond to repeated requests for information, and some diagnoses from healthcare providers did not confirm patient reports.
‡Statistically significant differences.

habitually engage in efforts to reduce risk, including use of repellents, protective clothing, tick checks, and yard management (*8*,*9*,*34*). In addition, awareness of relative risk might lead residents to spend more time in lawn and garden areas of their yards than in forested areas, where ticks were more abundant and the effects of treatments were stronger. These preventive behaviors could weaken the link between our tick-control interventions and disease incidence in the human population. If so, we would expect stronger effects of tick control in areas where residents demonstrate lower adherence to methods of personal protection. To examine this possibility, future studies could compare effectiveness of tick control interventions in areas of high and low adoption of personal protection measures.

The significant effect of active interventions observed for TBDs in outdoor pets but not in humans could have been caused by different patterns of space use (e.g., if outdoor pets spend more time in forested habitats within yards or use more of the neighborhood outside the individual property of residence). Use of repellents and other individual-based preventive measures might be less variable for pets than for humans, potentially increasing the ability to detect effects on pets. More information on how humans and pets use space, both within and outside residential areas, could help improve future tick-control interventions.

The observed effect of the active interventions on TBDs in outdoor pets should be interpreted cautiously. We observed no corresponding effect on tick encounters among pets, and we did not seek confirmation of pet diagnoses with veterinarians. Further, the incidence of TBDs in pets was the only outcome for which active Met52 treatments showed a significant effect.

In summary, although active TCS bait boxes were associated with reduced abundance of questing ticks, ticks attached to rodents, and TBD diagnoses in outdoor pets compared with placebo treatments, these interventions were not associated with significant reductions in human encounters with ticks or incidence of TBDs in humans. Thus, our study is consistent with that of Hinckley et al. (*23*) in suggesting that reducing the size of tick populations in residential areas might not result in strong effects on incidence of TBDs in human populations. More research is needed to address where in the environment, and under what conditions, humans most frequently encounter infected ticks, and in which geographic locations tick reductions will have the greatest impact on human health. One important conclusion for public health is that studies investigating tick reductions should also measure actual outcomes for people, such as disease incidence or tick encounters.

**Acknowledgments**

We are grateful for the support we received from the Steven & Alexandra Cohen Foundation; the Centers for Disease Control and Prevention; New York State; the Dutchess County Water and Wastewater Authority; the Ian Mactaggart Trust; the John Drulle, MD Memorial Lyme Fund, Inc.; the Pershing Square Foundation; The Walbridge Fund; Nina Brown deClercq; Susan and Jim Goodfellow; Elyse Harney; Eric Roberts; Pamela and Scott Ulm; and other private donors. We thank our partners: the Cary Institute of Ecosystem Studies, Bard College, the Centers for Disease Control and Prevention, the New York State Department of Health, and the Dutchess County Department of Community and Behavioral Health. The Cary Institute of Ecosystem Studies provided extensive logistical support. We benefited enormously from the expertise and dedication of the team at Arkus, Inc., especially Amy Bucciffero. Salesforce.org, Pardot, SMSMagic, FormAssembly, and DocUSign provided software and support at reduced fees. Our colleagues at Pestech Pest Solutions provided the professional applications of our treatments, under the committed leadership of Rob Robinson and Noah Startup. We are indebted to the staff of the Tick Project: Rose Adelizzi, Shefali Azad, Daniella Azulai, Kate Badour, Connor Baush, Mohammad Harris Bayan, Anna Butler, Reilly Carlson, Samantha Cassata, Adrian D'Souza, Dylan Dahan, Deanna DePietro, Joshua DiPaola, Lizzy Elliott, Michelle Ferrell, Amanda Gabryszak, Abigail Johnson, Amanda Jones, Alyssa Kamrowski, Ashley Kolton, Troy Koser, Beckett Lansbury, Rachel Livengood, Morgan Long, Sara McBride, Mica McCarty-Glen, Waynette McCracken, Sarah McGregor, Timothy McSweeny, Vince Meyer, Alison Molnar, Victoria Palfini, Nadine Pershyn, Nick Petterelli, Nicole Pierro, Luke Porter, Sophia Raithel, Madeline Rivard, Jared Russ, Samantha Sambado, Makayla Says, Megan Schierer, Brandi Strand, Katie Sweeney, Charlene Gray Tarsa, Abraham Turner, Nick Urbin, Agatha Winiarski, and Alex Wolf. The project benefited from the professional support of Josh Ginsberg, Holly Talbot, Lori Quillen, David Fischer, Amanda Johnson, Heather Malcom, Fred Merritt, Catherine Forbes, Ben Beard, Lars Eisen, Paul Mead, Jill Auerbach, Sue Serino, Didi Barrett, Robert S. Wills, and Sarah Dunphy-Lelii. This research was made possible by the generousparticipation of thousands of residents of Dutchess County, New York, and we extend our great appreciation to all of them. Two anonymous reviewers and an editor provided useful comments and suggestions that improved the manuscript.

This work was supported by a grant from the Steven & Alexandra Cohen Foundation, with additional financial and in-kind support from the Centers for Disease Control, New York State, the Dutchess County Water and Wastewater Authority, the Ian Mactaggart Trust, the John Drulle, MD Memorial Lyme Fund, Inc., the Pershing Square Foundation, The Walbridge Fund, Nina Brown deClercq, Susan and Jim Goodfellow, Elyse Harney, Eric Roberts, Pamela and Scott Ulm, and other private donors.

R.S.O. and F.K. designed the study. A.F.H., S.A.H., and A.S.E. provided input on study protocols. W.B., S.D., J.P., and M.T. collected data. F. Keating, A.P., and S.M. coordinated treatments and data. F.K. and S.M. analyzed the data. F.K. and R.S.O. wrote the first draft of the manuscript and all authors edited it.

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**Article DOI:** https://doi.org/10.3201/eid2805.211146

# Effects of Tick-Control Interventions on Tick Abundance, Human Encounters with Ticks, and Incidence of Tickborne Diseases in Residential Neighborhoods, New York

## Appendix

## Supplementary File: Detailed Methods

### Study Design

We tested whether 2 tick-control interventions, used separately or together, affected tick abundance, tick encounters with humans and their pets, and cases of tickborne diseases in humans and pets. Interventions were imposed on all participating residential properties within a neighborhood, so that the unit of replication was the residential neighborhood (exceptions for several statistical analyses are described later). Each intervention had a placebo control, further described below. Study participants and scientific personnel that collected or managed data on response variables were masked to treatment assignment. The assignment of neighborhoods to the 4 treatment groups was randomized. Hence, our design was randomized, placebo-controlled, and masked.

### Neighborhood Selection

We established 2 a priori criteria for selecting neighborhoods for inclusion in the study: (a) high incidence of Lyme disease (≈1% per year, which is 24× the overall incidence of Lyme disease for Dutchess County, New York, USA), and (b) moderate to high density of 1- and 2-family residences. The first criterion maximized the potential for detecting effects of interventions, and the second criterion increased feasibility of the research. We used georeferenced records of confirmed cases of Lyme disease reported during routine surveillance to the Dutchess County Department of Behavioral and Community Health (DCDBCH) during 2000–2011, totaling 8,100 cases. We selected 24 neighborhoods of ≈100 properties that contained a moderate to high density of 1- and 2-family homes and had high per capita Lyme

disease cases, while maintaining a minimum distance of 100 meters from other neighborhoods. Boundaries of neighborhoods were generally demarcated by physical features, such as large roads, commercial development, forested areas, or other nonresidential land-cover types.

### Participant Recruitment

We contacted residents of target properties through numerous outreach methods, including phone calls, door-to-door personal visits, the placement of study information and consent materials at doors, and door hangtags. All adults (≥18 years of age) were considered eligible to be the primary contact for a household if they lived in free-standing homes within target neighborhoods with a surrounding yard and the authority to allow treatments to be deployed on that property. We excluded properties when residents did not reside primarily at the property to be treated or were not willing to forgo the use of their own acaricides or insecticides on their yards during the study period.

Property recruitment began in April 2016 and continued until June 2017, after which no new properties were enrolled in the study. When participants moved from a property during the study, the new occupants of the property were given the opportunity to enroll if the change of occupancy occurred before September 2020.

### Introductory, Seasonal, and End-of-Study surveys

The primary contact for each participating household was asked to complete an introductory survey at the time of enrollment, during which they were asked to identify members of their households, as well as whether they had pets that spent time outside. The primary contact was also asked about prior diagnoses of tickborne diseases, outdoor activity levels and locations for all household members, adherence to preventive measures, and basic demographic information (including income, education, and age). We administered 4 additional seasonal surveys during the study to confirm that participants were still residing at properties. If participants responded affirmatively, we requested information about the number of persons and pets in residence at that time. At the conclusion of the study in December 2020, the primary contact was asked to complete a final survey, which included a question about whether they thought they knew the treatment group for their neighborhood.

**Treatments**

Beginning in spring 2017, we deployed 2 treatments, or their placebo equivalents, on participating properties: a spray containing spores of the F52 strain of the entomopathogenic fungus, *Metarhizium brunneum,* marketed as Met52® (Novozymes Biologicals, https://biosolutions.novozymes.com), and the Tick Control System (Select TCS, Tick Box Technology Corporation, http://www.tickboxtcs.com). TCS is a plastic box, which is placed inside a metal case that is staked to the ground. These boxes are designed to attract small mammals. Inside the box, the animals come into contact with a wick that applies the acaricide fipronil directly to their fur. Both interventions were selected on the basis of efficacy, as demonstrated in small-scale field trials (*1–5*), and commercial availability. The placebo control for Met52 was plain water, and the placebo control for TCS boxes were identical boxes that contained no fipronil.

For Met52 preparation for neighborhoods receiving active Met52 treatments, truck-mounted high-pressure sprayers (GNC Industries, Inc., https://gncindustries.com) were filled with 1,893 L (500 gallons) of water, into which Met52 spore suspension was added at a concentration of 2.22 L/378.5 L (100 gallons) of water and mixed thoroughly for a final concentration consistent with product label instructions. Identical trucks and sprayers were filled with water to treat the properties receiving placebo controls. All sprayers were filled at locations distant from the neighborhoods to be treated. Met52 or plain water was sprayed on all participating properties within designated neighborhoods at a pressure of 1.2– 1.4 mega Pascals (175–200 pounds per square inch), and at a rate of 4 L of spray per 93 $m^2$ (1,000 square feet). The sprayer apparatus, including the tank, was rinsed 3× with water after every neighborhood treatment. All vegetation on the property from ground level to 1 meter above the ground was sprayed according to product directions (e.g., avoiding water features). We also allowed residents to specify areas (e.g., vegetable gardens) not to be sprayed (n = 82 exclusion areas on 1,002 properties). In addition, when properties contained extensive forested areas, spraying extended 12 meters into the forest from the lawn or shrub and garden areas bordering the forest. Spraying was conducted twice each year to correspond to the periods immediately before and during the peak of questing activity of nymph-stage blacklegged ticks (*6*) (Appendix Table 1). We randomized the order in which each treatment group was sprayed (Appendix Table 1). Spraying was suspended during rain events and resumed within 24 hours thereafter.

The average property was treated with 5.9 (range 3–26) TCS boxes, or the equivalent number of placebo controls, which is a density of ≈38 boxes/hectare. Boxes were placed at least 10 meters apart in all habitat types that we sampled for ticks and were deployed in protected locations, such as along building foundations and under vegetation, that are frequently used by small mammals. Boxes were covered with galvanized steel shrouds and anchored in place with nylon zip ties attached to 31-cm long galvanized ground stakes (McGregor Fence Company, https://www.mcgregorfence1.com) to prevent disturbance by larger mammals. TCS boxes were deployed in 2 rounds each year, corresponding to the activity peaks for nymphal and larval blacklegged ticks (*6*), which ensured that the fipronil acaricide and bait were not depleted (Appendix Table 1).

#### Habitat Characterization

To determine habitat characteristics of the neighborhoods, we used publicly available statewide digital orthoimagery of Dutchess County (*7*) taken in the spring of 2016. We classified every pixel in each neighborhood by using the maximum-likelihood classification tool in ArcMap software version 10.4 (https://www.esri.com). To estimate forest habitat, we used a Geographic Information System (GIS) layer developed in 2017 by Robert S. Wills, Senior GIS Project Coordinator with Dutchess County Department of Planning and Development. We estimated error rates for all pixel classifications by selecting a random subset of 10% of the properties in each neighborhood and manually reviewing the output of the classification of the pixels on that property. The mean error rate for classification of lawn habitat by the maximum-likelihood classification tool was 12.9%. We considered this too high and opted instead to combine lawn/shrub/garden/field into 1 classification.

#### Tick Abundance: Questing Ticks

Each year, beginning in the late spring of 2017 through 2019, we sampled for ticks at 20 randomly selected properties within each neighborhood during the nymphal peak in blacklegged tick activity (*6*) in May–July. Sampling was not completed in 2020 because of the coronavirus disease pandemic. We sampled in 3 habitat types on each property: lawn, forest, and shrub or garden, whenever these habitat types were present. Because of variation in the size and arrangement of habitat features on properties, we performed timed flagging of each of the habitat types. We used 1-meter by 1-meter white corduroy cloth to flag-sample ticks modified from Rulison et al. (*8*), conducting up to ten 30-second intervals of flagging in each habitat type. We

avoided resampling and inspected the flagging cloth after each 30-second interval to count and collect all ticks. Researchers also counted and collected all ticks detected in self-inspections of field attire (white coveralls) at the end of each interval. Flagging was conducted between 9 AM and 5:30 PM each sampling day. We did not sample when it was raining or when vegetation was wet.

#### Tick Abundance: Tick Burdens on Small Mammals

To assess tick burdens on small mammals, we conducted mark-recapture sampling by using Sherman live traps (7.6 × 8.9 × 22.9 cm) at 10 randomly selected properties in each of the 24 neighborhoods from August through mid-to-late September annually during 2017–2019, corresponding to the activity peak of the larval stage (*6*). We set 18 traps on each property, with 2 traps at each of 9 trap stations and trap stations at least 5 meters apart, and checked them for 3 consecutive mornings. We baited traps with a 3:1 mixture of crimped oats and black oil sunflower seeds. We focused our trapping efforts and data collection on the white-footed mouse (*Peromyscus leucopus*) and the eastern chipmunk (*Tamias striatus*) because of their importance in transmitting the Lyme bacterium to ticks (*9*). We recorded the number of larval and nymphal ticks that we observed on an individual animal's head and ears, which prior research has demonstrated to accurately represent the total body burden (*10*). Each individual mouse or chipmunk was given a monel ear tag (style 1005–1, National Band & Tag Company, https://www.nationalband.com) with a unique identification number. Ticks were counted only upon the first capture of each individual animal. We released all animals at the point of capture, including nontarget species, which we released without handling.

#### Case and Encounter Data for Humans and Pets

To collect data on cases of tickborne diseases experienced by participating humans and their pets, and on human and pet encounters with ticks, we distributed biweekly surveys to each participating household. Every 2 weeks (Appendix Table 2), we contacted the primary contact person for each participating household to ask whether they or any other full-time resident of their household, including pets that spent time outdoors, had encountered a tick or had a diagnosis of a tickborne disease in the previous 2 weeks. If a participant answered yes to the initial question, they were prompted to complete a follow-up survey.

Follow-up surveys requested further information on human or pet encounters with ticks. Participants reporting tickborne illness in a person were asked to consider signing a medical consent form and completing a Health Insurance Portability and Accountability Act (HIPAA) release enabling us to request confirmation of the case by their healthcare provider. If we received a completed medical consent and HIPAA release, designated staff members contacted the healthcare provider's office to request an abstraction of the diagnosis.

#### Informed Consent

The Institutional Review Board of the Cary Institute of Ecosystem Studies in Millbrook, NY, USA reviewed and approved protocols (approval no. 131–2016) involving human subjects. All participants were fully informed of study procedures, their legal rights and responsibilities, the general scientific benefits expected from the research, and their right for voluntary termination without penalty or censure.

#### Animal Care and Handling

Protocols for the live-trapping and handling of small mammals were approved by the Cary Institute Institutional Animal Care and Use Committee (IACUC Code 02–16). Trapping was conducted under License to Collect or Possess: Scientific #1512 from the New York State Department of Environmental Conservation.

### Data Analysis

Data on participants were stored in Salesforce (https://www.salesforce.com), with password-protected access that masked confidential and sensitive information. Access to data was provided to personnel only as needed, and all persons who collected, entered, or worked with raw data were unaware of treatment assignments. Field data on ticks and rodents were recorded in Microsoft Excel (https://www.microsoft.com). Data were analyzed in R version 4.0.1 (*11*), using the packages *tidyr* (*12*), *dplyr* (*13*), and *forcats* (*14*) to format and manipulate data, and the packages *ggplot2* (*15*) and *cowplot* (*16*) for graphing. We used the *broom* (*17*) and *broom.mixed* (*18*) packages to tidy statistical output. We analyzed data on the proportion of each neighborhood that comprised forest and nonforest (i.e., shrub/lawn/garden) habitat by using linear models, with treatment as a factor, checking that assumptions of tests were met.

**Tick Abundance: Questing Ticks**

We analyzed data on the abundance of questing nymphal ticks in May–early July over a 3-year period beginning in 2017, the year treatments were first applied to the properties. Tick densities were estimated as the mean number of questing nymphal ticks per flagging interval, with each habitat type (forest, lawn, shrub/garden) evaluated separately. We sampled ticks twice on each property during each year's nymphal activity period, and we estimated tick density on a property as the maximum of the 2 samples. For 12 of 10,778 occasions, the number of intervals in a particular habitat type on a particular property was not recorded. In these cases, for the particular habitat type, we used the number of intervals recorded during the second visit of the year to the same property.

We evaluated our data on questing ticks in 2 ways. First, we analyzed data at the neighborhood level, using the mean of the maximum number of questing nymphal ticks collected per interval from each sampled property within a neighborhood, averaged for each neighborhood. Because we sampled the same neighborhoods over 3 years, we treated neighborhood as a random effect, with year, treatment, and an interaction between the presence of active TCS boxes and active Met52 treatments as fixed effects. We used linear mixed-effects models built by using package *nlme* (*19*) to conduct these analyses, transforming the data when needed to conform to assumptions of tests.

In our second analysis, we analyzed data from each of the individual properties we sampled, without averaging these data for the entire neighborhood. For these property-level analyses, we only included data from properties at which we were able to conduct fieldwork in all 3 years: 2017, 2018, and 2019. Each neighborhood had a minimum of 10 properties with 3 complete years of sampling (mean 15.8 $\pm$ 0.6 SEM). For flagging data, zero values were common, representing 43%–80% of our property-level data, depending on habitat type. To evaluate these data at the property level, we used generalized linear mixed models with a binomial distribution and a logit link function to fit a logistic model to the zero values versus the nonzero values. These analyses were conducted with the *glmmTMB* function in package *glmmTMB* (*20*). We tested whether our data met the assumptions of our models by using the *DHARMa* package (*21*). We did not attempt to fit a generalized linear model to the nonzero values, as in a hurdle modeling approach, because the nonzero values did not conform to the assumptions of distributions (e.g., gamma, gaussian).

### Tick Abundance: Tick Burdens on Small Mammals

For data on the tick burdens on white-footed mice (*Peromyscus leucopus*) and eastern chipmunks (*Tamias striatus*), we calculated the weighted mean of the number of larval and nymphal ticks on each species on each property by using the number of unique individual animals (mice or chipmunks) on each property as the weighting factor. More than 98% of ticks were larvae, with <2% nymphs. Means of tick burdens did not include any small mammals that escaped during handling or any small mammals that had been previously trapped at a different property within that season. Some properties in a neighborhood had no captures of mice or no captures of chipmunks in a given year. For these properties, there was no estimate of ticks on that species of rodent, and these properties were excluded from calculations of that neighborhood's mean tick burdens for that year.

Because we sampled the same neighborhoods over 3 years, we treated neighborhood as a random effect in our statistical models, with year, treatment, and an interaction between the presence of active TCS boxes and active Met52 treatments as fixed effects. The weighted means of ticks on mice were analyzed by using linear mixed effects models with package *nlme* (*19*), using a square-root transformation of the data to conform to assumptions of tests. The weighted means of ticks on chipmunks did not conform to the assumptions of linear models, so we first used a generalized linear mixed model with a binomial distribution and a logit link function to fit a logistic model to the zero values versus the nonzero values. We then used a generalized linear mixed model with a gamma distribution for the nonzero values, with neighborhood as a random effect, and year, treatment, and an interaction between the use of active TCS boxes and/or Met52 spray as fixed effects. These analyses were conducted with the *glmmTMB* function in package *glmmTMB* (*20*). We tested whether our data met the assumptions of the models by using the *DHARMa* package (*21*).

### Case and Encounter Data for Humans and Pets

Our data for encounters with ticks and cases of tickborne diseases for both humans and pets were gathered from a total of 78 biweekly surveys, which we administered to participants during May 2017–December 2020 (Appendix Table 2). For all case and encounter data, we included in our analyses estimates of the number of participating persons or pets in a neighborhood over a particular year. The number of persons and pets was determined by surveys conducted at the start or end of each field season. If a participating household did not respond to

a specific survey, we assumed the number of persons and pets remained the same as in the previous survey. We used these data to calculate the number of human and pet participants in a neighborhood during each biweekly survey period and then averaged these biweekly estimates to establish the annual number of participants. If participants informed us of changes to the number of residents or pets in their household during a season, these changes were incorporated at the time of the relevant biweekly survey. Members of households that withdrew from the study were not included in analyses of cases for seasons in which they were enrolled for fewer than half of the biweekly surveys.

We calculated the cumulative number of reported encounters by humans or pets for all participating households in a neighborhood on the basis of participant responses to our biweekly surveys. A specific tick encounter reported by a participant was calculated as a binary value (yes/no), regardless of the number of ticks the participant reported having encountered. To be included in a particular year's data on encounters or cases, a household needed to have responded to $\geq$1 of that year's biweekly surveys.

We analyzed the number of human cases in 2 ways. First, we considered self-reported cases of tickborne diseases indicated by participants during biweekly surveys. For these self-reports, participants were asked to report household cases of tickborne diseases diagnosed by a healthcare provider. When participants granted us permission to contact their healthcare provider, we did so, using the clinical diagnosis of a case as indicated by the healthcare provider as the basis for a confirmed case. For cases in pets, we relied on reports of diagnosed cases from owners through the biweekly surveys.

For reported tick encounters and cases of tickborne diseases for humans and pets, we calculated the cumulative number of reports of each type for each neighborhood for the 4 years of treatments. Because our data relied on responses to our biweekly surveys, our estimates of the number of participants in a neighborhood included only participants or pets from households that submitted $\geq$1 biweekly survey in a given year. We used generalized linear models with treatment and an interaction between the presence of active TCS boxes and active Met52 as fixed effects, with an offset for the mean number of humans (or pets) in a neighborhood. We used the *glmmTMB* function in package *glmmTMB* (*20*) to fit models with a negative binomial distribution. We evaluated the residuals of the models (e.g., for overdispersion) by using the

*DHARMa* package (*21*). When necessary to account for a large number of zero values in a particular response variable, we included a term for zero-inflation.

## Supplementary Results

### Study Participants

When the study began, a mean of 101 persons (range 62–136) and 35 outdoor pets (range 14–58) were enrolled in each neighborhood, for a total of 2,384 human participants and 849 pets. Because of attrition, an average of 7% fewer households were enrolled by the end of the study, reaching a mean of 95 participants per neighborhood (range 61–124) (Appendix Figure 2). Approximately two thirds of the primary contacts for each household reported educational attainment of a college degree or higher (Appendix Figure 3).

Approximately 80% of participating households responded to the biweekly survey regarding tick encounters and disease diagnoses, and 83% responded to at least half of the surveys each season. Ninety-six percent responded to $\geq$1 of the biweekly surveys in any given season.

### Effectiveness of Masking Procedures

Of 874 households participating in December 2020, 507 primary contacts (58%) completed the final survey and 438 of these (86%) said they did not know their neighborhood's treatment assignment. Of the 65 that thought they knew their neighborhood's treatment assignment, their guesses were incorrect (54%) more frequently than they were correct (46%). Of the 13% (N = 65) who said they thought they knew $\geq$1 of the treatment assignments, most thought they knew either the type of Met52 treatment they received (43%) or the types of both treatments (40%). Only 17% thought they knew only the type of bait boxes their neighborhood was assigned. Of those who thought they knew the type of Met52 treatment they received, 15 of 28 (53%) were correct, and there was no statistically significant association between their guess and their actual treatment category based on a Fisher exact test ($\chi^2 = 0.53$; $p>0.05$).

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**Appendix Table 1.** Dates of tick-control treatments for both TCS boxes and Met52 spray, New York, 2017–2020*

| Year | Order | TCS box and Met52 deployment | Met52 | TCS box pick up and deployment | TCS box pick up |
|---|---|---|---|---|---|
| 2017 | *a-b-c-d* | 12 April–5 May | 1–28 June | 5 July–4 Aug | 19 Oct–17 Nov |
| 2018 | *d-c-b-a* | 12 April–8 May | 1–27 June | 5 July–1 Aug | 5–31 Oct |
| 2019 | *c-d-a-b* | 12 April–13 May | 3–28 June | 5–31 July | 7–31 Oct |
| 2020 | *b-d-a-c* | 13 April–18 May | 1–30 June | 6–31 July | 5–29 Oct |

*To avoid bias in the sequence of visitation for each treatment category, neighborhoods in the 4 treatment groups (± active TCS boxes and ± active Met52 spray) were visited in a different order each year, as indicated in the second column. TCS, Tick Control System.

**Appendix Table 2.** Annual number of biweekly surveys in study of tick-control interventions in residential neighborhoods, New York, with start and end dates, 2017–2020

| Year | No. biweekly surveys | First biweekly survey | Last biweekly survey |
|---|---|---|---|
| 2017 | 15 | 2 May | 14 November |
| 2018 | 19 | 27 March | 4 December |
| 2019 | 22 | 19 February | 10 December |
| 2020 | 22 | 18 February | 8 December |

**Appendix Table 3.** Results of linear model of the proportion of households in each neighborhood that were enrolled in study of tick-control interventions in residential neighborhoods, New York, 2017, with treatment as a factor*

| Term | Estimate | SE | t statistic | p value |
|---|---|---|---|---|
| (Intercept) | 0.35 | 0.02 | 15.02 | 0.00 |
| Active Met52 | 0.00 | 0.03 | −0.01 | 0.99 |
| Active TCS boxes | −0.01 | 0.03 | −0.41 | 0.69 |
| Active TCS boxes and active Met52 | −0.02 | 0.03 | −0.63 | 0.53 |

*TCS, Tick Control System.

**Appendix Table 4.** Results of linear model of proportion of each neighborhood comprised of forest in study of tick-control interventions in residential neighborhoods, New York, with treatment as a factor*

| Term | Estimate | SE | t statistic | p value |
|---|---|---|---|---|
| (Intercept) | 0.46 | 0.05 | 9.06 | <<0.01 |
| Active Met52 | −0.02 | 0.07 | −0.21 | 0.84 |
| Active TCS boxes | 0.012 | 0.07 | 0.16 | 0.87 |
| Active TCS boxes and active Met52 | −0.09 | 0.07 | −1.19 | 0.25 |

*TCS, Tick Control System.

**Appendix Table 5.** Results of linear model of proportion of each neighborhood comprised of non-forest habitat (lawn, shrub, garden) in study of tick-control interventions in residential neighborhoods, New York, with treatment as a factor*

| Term | Estimate | SE | t statistic | p value |
|---|---|---|---|---|
| (Intercept) | 0.29 | 0.03 | 8.30 | 0.00 |
| Active Met52 | 0.01 | 0.05 | 0.24 | 0.81 |
| Active TCS boxes | −0.02 | 0.05 | −0.41 | 0.69 |
| Active TCS boxes x Met52 | 0.06 | 0.05 | 1.13 | 0.27 |

*TCS, Tick Control System.

**Appendix Table 6.** Results of linear mixed-effects model of the average maximum number of questing nymphal ticks per interval in forested habitat, New York, 2017–2019, analyzed at the neighborhood level, with year, treatments, and an interaction between active TCS boxes and active Met52 as fixed effects and neighborhood as a random factor*

| Effect | Group | Term | Estimate | SE | df | t value | p value |
|---|---|---|---|---|---|---|---|
| Fixed | Fixed | (Intercept) | 82.05 | 29.16 | 47.00 | 2.81 | 0.01 |
| Fixed | Fixed | Year | −0.04 | 0.01 | 47.00 | −2.80 | 0.01* |
| Fixed | Fixed | Active TCS boxes | −0.20 | 0.09 | 20.00 | −2.24 | 0.04* |
| Fixed | Fixed | Active Met52 | −0.15 | 0.09 | 20.00 | −1.64 | 0.12 |
| Fixed | Fixed | Active TCS boxes and active Met52 | 0.17 | 0.13 | 20.00 | 1.36 | 0.19 |
| Random | Neighborhood | sd (Intercept) | 0.14 | | | | |
| Random | Residual | sd Observation | 0.10 | | | | |

*TCS, Tick Control System.

**Appendix Table 7**. Results of linear mixed-effects model of the average maximum number of questing nymphal ticks per interval in shrub and garden habitat, New York, 2017–2019, analyzed at the neighborhood level, with year, treatments, and an interaction between active TCS boxes and active Met52 as fixed effects and neighborhood as a random factor*

| Effect | Group | Term | Estimate | SE | df | t value | p value |
|---|---|---|---|---|---|---|---|
| Fixed | fixed | (Intercept) | −43.66 | 19.19 | 47.00 | −2.27 | 0.03 |
| Fixed | fixed | Year | 0.02 | 0.01 | 47.00 | 2.29 | 0.03* |
| Fixed | fixed | Active TCS boxes | −0.08 | 0.04 | 20.00 | −2.15 | 0.04* |
| Fixed | fixed | Active Met52 | −0.05 | 0.04 | 20.00 | −1.34 | 0.20 |
| Fixed | fixed | Active TCS boxes x Met52 | 0.06 | 0.05 | 20.00 | 1.09 | 0.29 |
| Random | Neighborhood | sd (Intercept) | 0.05 | | | | |
| Random | Residual | sd Observation | 0.07 | | | | |

*TCS, Tick Control System.

**Appendix Table 8.** Results of linear mixed-effects model of the average maximum number of questing nymphal ticks per interval in lawn habitat, New York, 2017–2019, analyzed at the neighborhood level, with year, treatments, and an interaction between active TCS boxes and active Met52 as fixed effects and neighborhood as a random factor*

| Effect | Group | Term | Estimate | SE | df | t value | p value |
|---|---|---|---|---|---|---|---|
| Fixed | fixed | (Intercept) | −13.79 | 15.48 | 47.00 | −0.89 | 0.38 |
| Fixed | fixed | Year | 0.01 | 0.01 | 47.00 | 0.90 | 0.37 |
| Fixed | fixed | Active TCS boxes | −0.06 | 0.04 | 20.00 | −1.70 | 0.10 |
| Fixed | fixed | Active Met52 | −0.04 | 0.04 | 20.00 | −1.06 | 0.30 |
| Fixed | fixed | Active TCS boxes x Met52 | 0.03 | 0.05 | 20.00 | 0.55 | 0.59 |
| Random | Neighborhood | sd (Intercept) | 0.05 | | | | |
| Random | Residual | sd Observation | 0.05 | | | | |

*TCS, Tick Control System.

**Appendix Table 9**. Results of generalized linear mixed-effects model of the detection of questing nymphal ticks in forest habitat, New York, 2017–2019, analyzed at the property level, with year, treatments, and an interaction between active TCS boxes and active Met52 as fixed effects, and property within neighborhood as a random effect*

| Effect | Group | Term | Estimate | SE | z value | p value |
|---|---|---|---|---|---|---|
| Fixed | | (Intercept) | 1.59 | 0.49 | 3.24 | 0.00 |
| Fixed | | Year | −0.08 | 0.09 | −0.82 | 0.41 |
| Fixed | | Active TCS boxes | −1.68 | 0.64 | −2.64 | 0.01* |
| Fixed | | Active Met52 | −1.14 | 0.64 | −1.78 | 0.07 |
| Fixed | | Active TCS boxes x Met52 | 1.10 | 0.90 | 1.22 | 0.22 |
| Random | Property | sd (Intercept) | 1.18 | | | |
| Random | Neighborhood | sd (Intercept) | 0.99 | | | |

*TCS, Tick Control System.

**Appendix Table 10**. Results of generalized linear mixed-effects model of the detection of questing nymphal ticks in shrub and garden habitat, New York, 2017–2019, analyzed at the property level, with year, treatments, and an interaction between active TCS boxes and active Met52 as fixed effects, and property within neighborhood as a random effect*

| Effect | Group | Term | Estimate | SE | z value | p value |
|---|---|---|---|---|---|---|
| Fixed | | (Intercept) | −1.60 | 0.33 | −4.91 | 0.00 |
| Fixed | | Year | 0.30 | 0.09 | 3.20 | 0.00* |
| Fixed | | Active TCS boxes | −1.30 | 0.38 | −3.37 | 0.00* |
| Fixed | | Active Met52 | −0.59 | 0.37 | −1.61 | 0.11 |
| Fixed | | Active TCS boxes x Met52 | 0.85 | 0.54 | 1.56 | 0.12 |
| Random | Property | sd (Intercept) | 0.73 | | | |
| Random | Neighborhood | sd (Intercept) | 0.50 | | | |

*TCS, Tick Control System.

**Appendix Table 11**. Results of generalized linear mixed-effects model of the detection of questing nymphal ticks in lawn habitat, New York, 2017–2019, analyzed at the property level, with year, treatments, and an interaction between active TCS boxes and active Met52 as fixed effects, and property within neighborhood as a random effect*

| Effect | Group | Term | Estimate | SE | z value | p value |
|---|---|---|---|---|---|---|
| Fixed | | (Intercept) | −1.08 | 0.36 | −3.00 | 0.00 |
| Fixed | | Year | 0.06 | 0.09 | 0.71 | 0.48 |
| Fixed | | Active TCS boxes | −1.00 | 0.45 | −2.22 | 0.03* |
| Fixed | | Active Met52 | −0.51 | 0.44 | −1.15 | 0.25 |
| Fixed | | Active TCS boxes x Met52 | 0.48 | 0.64 | 0.75 | 0.45 |
| Random | Property | sd (Intercept) | 0.77 | | | |
| Random | Neighborhood | sd (Intercept) | 0.66 | | | |

*TCS, Tick Control System.

**Appendix Table 12**. Results of linear mixed-effects model of the weighted mean number of ticks on white-footed mice, New York,with year, treatment, and an interaction between active TCS boxes and active Met52 as fixed effects and neighborhood as a random effect*

| Effect | Group | Term | Estimate | SE | df | t value | p value |
|---|---|---|---|---|---|---|---|
| Fixed | fixed | (Intercept) | −340.05 | 222.00 | 47 | −1.53 | 0.13 |
| Fixed | fixed | Year | 0.17 | 0.11 | 47 | 1.54 | 0.13 |
| Fixed | fixed | Active TCS boxes | −0.80 | 0.34 | 20 | −2.37 | 0.03* |
| Fixed | fixed | Active Met52 | −0.26 | 0.34 | 20 | −0.77 | 0.45 |
| Fixed | fixed | Active TCS boxes x Met52 | 0.26 | 0.48 | 20 | 0.55 | 0.59 |
| Random | Neighborhood | sd_(Intercept) | 0.39 | | | | |
| Random | Residual | sd_Observation | 0.76 | | | | |

*TCS, Tick Control System.

**Appendix Table 13**. Results of hurdle modeling of the weighted mean of the number of ticks on chipmunks, New York*

| | | | | | | |
|---|---|---|---|---|---|---|
| Zero versus nonzero values of the weighted mean of ticks on chipmunks† | | | | | | |
| Fixed | | (Intercept) | 23.06 | 21235.89 | 0.00 | 1.00 |
| Fixed | | 2018 | 2.77 | 1.48 | 1.87 | 0.06 |
| Fixed | | 2019 | 2.77 | 1.48 | 1.87 | 0.06 |
| Fixed | | Active TCS boxes | −23.46 | 21235.89 | 0.00 | 1.00 |
| Fixed | | Active Met52 | −19.92 | 21235.89 | 0.00 | 1.00 |
| Fixed | | Active TCS boxes x Met52 | 19.22 | 21235.89 | 0.00 | 1.00 |
| Random | Neighborhood | sd__(Intercept) | 2.19 | | | |
| Positive values of the weighted mean of ticks on chipmunks‡ | | | | | | |
| Fixed | | (Intercept) | −0.23 | 0.33 | −0.70 | 0.49 |
| Fixed | | 2018 | −0.28 | 0.29 | −0.97 | 0.33 |
| Fixed | | 2019 | 0.25 | 0.30 | 0.83 | 0.41 |
| Fixed | | Active TCS boxes | −0.12 | 0.44 | −0.27 | 0.79 |
| Fixed | | Active Met52 | 0.01 | 0.41 | 0.02 | 0.98 |
| Fixed | | Active TCS boxes x Met52 | −0.18 | 0.63 | −0.28 | 0.78 |
| Random | Neighborhood | (Intercept) | 0.50 | | | |

*TCS, Tick Control System.
†Results of generalized linear model of the zero versus nonzero values for the weighted means, fit to a logistic distribution with year and an interaction between active TCS boxes and active Met52 as fixed effects and neighborhood as a random effect.
‡Results of generalized linear model of the nonzero values for the weighted means, fit with a gamma distribution, with year and an interaction between active TCS boxes and active Met52 as fixed effects and neighborhood as a random effect.

**Appendix Table 14.** Results of generalized linear model of cumulative self-reported encounters with ticks by human participants in tick-control intervention study, New York, 2017–2020, fit with a negative binomial distribution and an offset for the number of human participants in a neighborhood*

| Term | Estimate | SE | z value | p value |
|---|---|---|---|---|
| (Intercept) | −0.34 | 0.15 | −2.24 | 0.03 |
| Active Met52 | 0.01 | 0.22 | 0.05 | 0.96 |
| Active TCS boxes | 0.15 | 0.21 | 0.73 | 0.47 |
| Active TCS boxes x Met52 | −0.38 | 0.32 | −1.17 | 0.24 |

*TCS, Tick Control System.

**Appendix Table 15.** Results of generalized linear model of cumulative self-reported cases of tickborne diseases in humans, New York, 2017–2020, fit with a negative binomial distribution and an offset for the number of human participants in a neighborhood*

| Term | Estimate | SE | z value | p value |
|---|---|---|---|---|
| (Intercept) | −3.07 | 0.21 | −14.53 | 0.00 |
| Active Met52 | 0.09 | 0.30 | 0.30 | 0.76 |
| Active TCS boxes | 0.30 | 0.29 | 1.02 | 0.31 |
| Active TCS boxes x Met52 | −0.05 | 0.41 | −0.11 | 0.91 |

*TCS, Tick Control System.

**Appendix Table 16**. Results of generalized linear model of cumulative cases of tickborne diseases in humans, New York, 2017–2020, as confirmed by a healthcare provider*

| Term | Estimate | SE | z value | p value |
|---|---|---|---|---|
| (Intercept) | −4.68 | 0.45 | −10.31 | 0.00 |
| Active Met52 | 0.36 | 0.60 | 0.59 | 0.55 |
| Active TCS boxes | 0.82 | 0.57 | 1.43 | 0.15 |
| Active TCS boxes x Met52 | −0.59 | 0.79 | −0.75 | 0.46 |

*The model is fit with a negative binomial distribution and an offset for the number of participating persons in a neighborhood. TCS, Tick Control System.

**Appendix Table 17.** Results of generalized linear model of cumulative pet encounters with ticks, New York, 2017–2020, fit with a negative binomial distribution and an offset for the number of participating pets in a neighborhood*

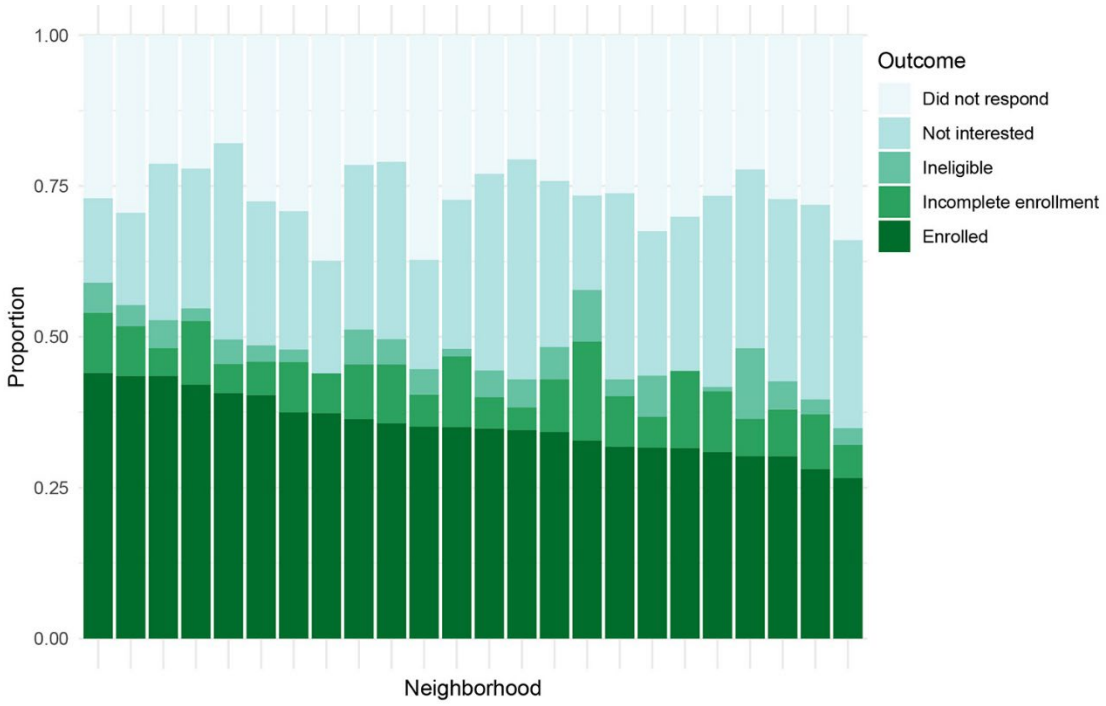
| Term | Estimate | SE | z value | p value |
|---|---|---|---|---|
| (Intercept) | 0.59 | 0.16 | 3.79 | 0.00 |
| Active Met52 | −0.08 | 0.22 | −0.35 | 0.73 |
| Active TCS boxes | 0.14 | 0.20 | 0.69 | 0.49 |
| Active TCS boxes x Met52 | −0.29 | 0.30 | −0.96 | 0.34 |

*TCS, Tick Control System.

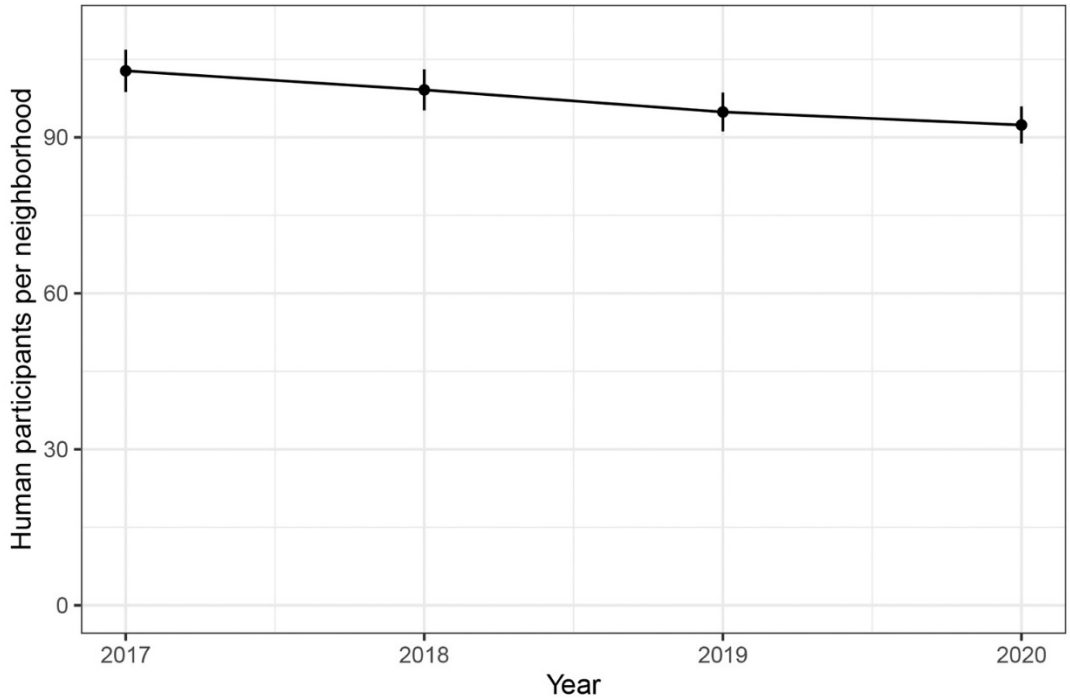
**Appendix Table 18**. Results of generalized linear model of cumulative diagnosed cases of tickborne diseases in pets, New York, 2017–2020, fit with a Poisson distribution and with an offset for the number of participating pets in a neighborhood*

| Term | Estimate | SE | z value | p value |
|---|---|---|---|---|
| (Intercept) | −1.81 | 0.19 | −9.56 | 0.00 |
| Active Met52 | −0.67 | 0.32 | −2.11 | 0.04* |
| Active TCS boxes | −0.70 | 0.31 | −2.25 | 0.02* |
| Active TCS boxes x Met52 | 0.76 | 0.47 | 1.61 | 0.11 |

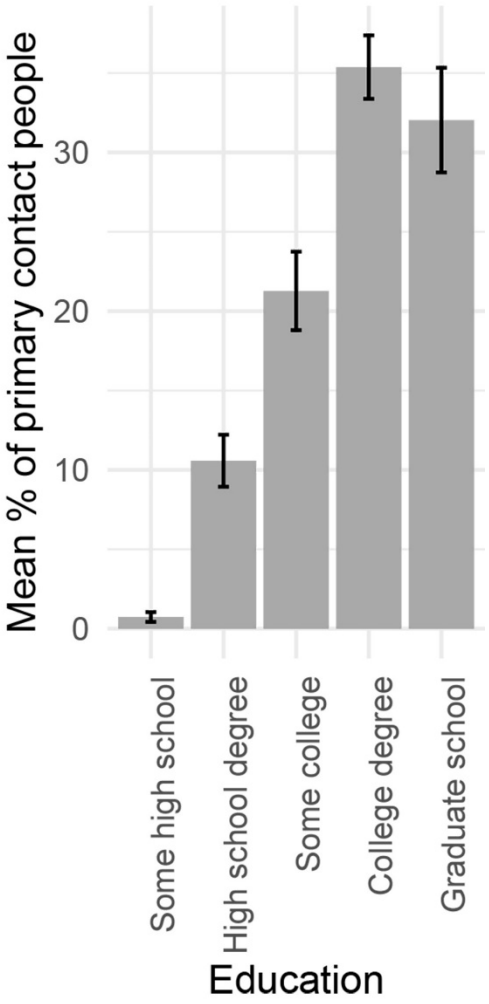
*TCS, Tick Control System.



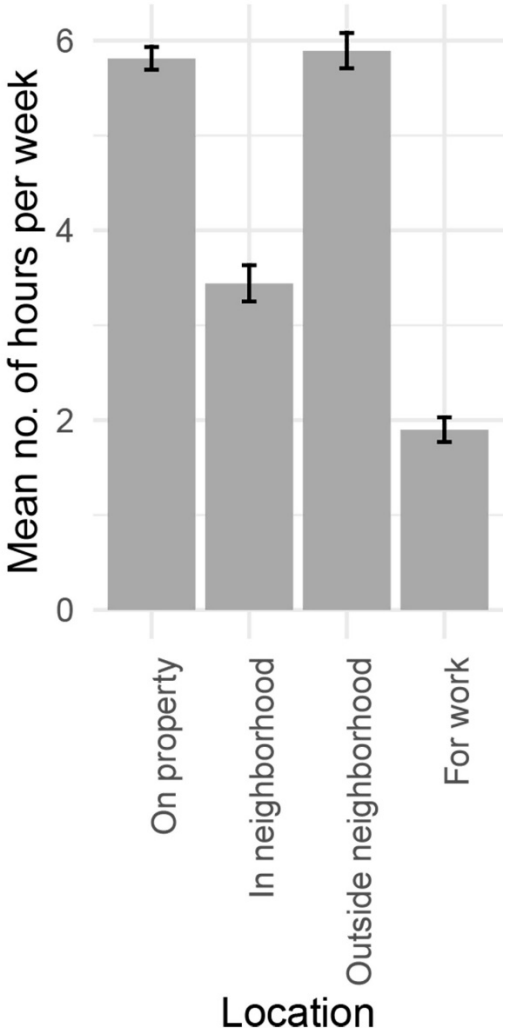

**Appendix Figure 1**. Proportion of households in each residential neighborhood with specified recruitment outcomes in study of tick-control interventions, New York. Bars represent each of the 24 neighborhoods in order of declining participation. Households were saturated with contacts, including mailings, door-tags, in-person visits, and, when phone numbers were available, phone calls.




**Appendix Figure 2**. Mean number of human participants enrolled in study of tick-control interventions in each residential neighborhood, New York, 2017–2020. Error bars represent standard errors.




**Appendix Figure 3**. Mean percentage of primary contact people in each residential New York neighborhood participating in study of tick-control interventions who had the indicated level of educational attainment, averaged across the 24 neighborhoods. Error bars represent standard errors.




**Appendix Figure 4**. Mean number of hours spent outside per week by tick-control study participants in locations in or away from the neighborhood, based on responses to the enrollment survey administered in 2016–2017. Error bars represent standard errors.