Journal of Medical Entomology, 59(6), 2022, 2080–2089
https://doi.org/10.1093/jme/tjac106
Advance Access Publication Date: 18 August 2022
Research

ENTOMOLOGICAL SOCIETY OF AMERICA
SHARING INSECT SCIENCE GLOBALLY

OXFORD

Vector Control, Pest Management, Resistance, Repellents

Fluralaner Baits Reduce the Infestation of *Peromyscus* spp. Mice (Rodentia: Cricetidae) by *Ixodes scapularis* (Acari: Ixodidae) Larvae and Nymphs in a Natural Environment

Jérôme Pelletier,[1,2,3,7] Jean-Philippe Rocheleau,[2,4] Cécile Aenishaenslin,[1,2,3] Gabrielle Dimitri Masson,[1,2] L. Robbin Lindsay,[5] Nicholas H. Ogden,[2,6] Catherine Bouchard,[2,6] and Patrick A. Leighton[1,2,3]

[1]Département de pathologie et microbiologie, Faculté de médecine vétérinaire, Université de Montréal, Saint-Hyacinthe, Québec, Canada [2]Groupe de recherche en épidémiologie des zoonoses et santé publique, Faculté de médecine vétérinaire, Université de Montréal, Saint-Hyacinthe, Québec, Canada [3]Centre de recherche en santé publique de l'Université de Montréal et du CIUSSS du Centre-Sud-de-l'Île-de-Montréal, Montréal, Québec, Canada [4]Département de santé animale, CÉGEP de Saint-Hyacinthe, Saint-Hyacinthe, Québec, Canada [5]One Health Division, National Microbiology Laboratory, Public Health Agency of Canada, Winnipeg, Manitoba, Canada [6]Public Health Risk Sciences Division, National Microbiology Laboratory, Public Health Agency of Canada, Saint-Hyacinthe, Québec, Canada and [7]Corresponding author, e-mail: jerome.pelletier.1@umontreal.ca

Subject Editor: Stephen Rich

Received 13 March 2022; Editorial decision 5 July 2022.

Abstract

The development of interventions that reduce Lyme disease incidence remains a challenge. Reservoir-targeted approaches aiming to reduce tick densities or tick infection prevalence with *Borrelia burgdorferi* have emerged as promising ways to reduce the density of infected ticks. Acaricides of the isoxazoline family offer high potential for reducing infestation of ticks on small mammals as they have high efficacy at killing feeding ticks for a long period. Fluralaner baits were recently demonstrated as effective, in the laboratory, at killing *Ixodes scapularis* larvae infesting *Peromyscus* mice, the main reservoir for *B. burgdorferi* in north-eastern North America. Here, effectiveness of this approach for reducing the infestation of small mammals by immature stages of *I. scapularis* was tested in a natural environment. Two densities of fluralaner baits (2.1 baits/1,000 m^2 and 4.4 baits/1,000 m^2) were used during three years in forest plots. The number of *I. scapularis* larvae and nymphs per mouse from treated and control plots were compared. Fluralaner baiting reduced the number of larvae per mouse by 68% (CI95: 51–79%) at 2.1 baits/1,000 m^2 and by 86% (CI95: 77–92%) at 4.4 baits/1,000 m^2. The number of nymphs per mouse was reduced by 72% (CI95: 22–90%) at 4.4 baits/1,000 m^2 but was not significantly reduced at 2.1 baits/1,000 m^2. Reduction of *Peromyscus* mouse infestation by immature stages of *I. scapularis* supports the hypothesis that an approach targeting reservoirs of *B. burgdorferi* with isoxazolines has the potential to reduce tick-borne disease risk by decreasing the density of infected ticks in the environment.

Key words: Lyme disease, *Borrelia burgdorferi*, *Ixodes scapularis*, *Peromyscus*, fluralaner

In central and eastern North America, Lyme disease (LD) risk is driven by the presence of blacklegged ticks, *Ixodes scapularis* Say, infected with the bacterium *Borrelia burgdorferi sensu stricto* (here after *B. burgdorferi*) in the environment (DIT) (Mather et al. 1989, Kitron and Kazmierczak 1997, Pepin et al. 2012).

LD represents an important burden on public health. It is associated with a loss of 84.5 quality-adjusted life years per 100,000 people in Canada (Mac et al. 2021). The economic burden of this disease is also substantial due to high direct and indirect medical costs (Zhang et al. 2006, Mac et al. 2019). In addition, recent studies

in the northeast United States (Berry et al. 2018) and southern Canada (Aenishaenslin et al. 2021) show that individuals spent less time in outdoor activities in response to perceived LD risk, which can impact on their health and quality of life. This burden is expected to increase in temperate areas of the northern hemisphere where the geographic distribution of LD is still expanding, such as Canada or northern Europe (Ogden et al. 2009, Leighton et al. 2012, Bouchard et al. 2015, Vandekerckhove et al. 2019). Duration and frequency of outdoor activities in public or in peridomestic woodlands with a high DIT are important risk factors for human exposure to ticks infected with *B. burgdorferi* (Smith et al. 1988, Lane et al. 1992, Ley et al. 1995, Mead et al. 2018, Aenishaenslin et al. 2022). The risk of human-tick encounters resulting in LD is greatest when people engage in outdoor activities within a peridomestic event (i.e., within their own yard or residential neighborhood (Fischhoff et al. 2019).

The ongoing emergence of LD risk makes the development of effective interventions to prevent human exposure to infected ticks a public health priority. However, to date, few interventions showed effectiveness at reducing LD incidence despite the development of many different approaches over the past decades (Eisen 2021). Preventive interventions aiming to reduce *B. burgdorferi* transmission to humans fall into four strategic objectives: 1) reducing tick density in the environment, 2) lowering prevalence of *B. burgdorferi* infection in the reservoir host and thus in host-seeking *B. burgdorferi*-infected ticks, 3) modifying human behavior to reduce either exposure to infected ticks or the probability of pathogen transmission, and 4) preventing infection through either vaccination or postexposure prophylactic antibiotic treatment (Eisen et al. 2012). Strategies 1 and 2 aim to reduce the DIT which in turn would reduce the risk of LD. Targeting reservoir hosts of *B. burgdorferi*, such as small mammals, has the potential to meet strategic objectives 1 and 2 in one approach, because these hosts feed a high proportion of *I. scapularis* larvae and nymphs (Eisen and Eisen 2018).

In eastern and central North America, the most important reservoir hosts to target are mice within the genus *Peromyscus*; namely: the white-footed mouse, *Peromyscus leucopus* Rafinesque, and the deer mouse, *Peromyscus maniculatus* Wagner (Levine et al. 1985, Mather et al. 1989, Rand et al. 1993, Bouchard et al. 2011). Ticks can maintain *B. burgdorferi* trans-stadially but this pathogen is not transmitted vertically from adult female *I. scapularis* to their eggs or subsequent life stages (Patrican 1997, Scoles et al. 2001). Thus, reservoir hosts play a key role in *B. burgdorferi* endemic cycles by transmitting and amplifying the infection they acquire from infected *I. scapularis* nymphs to current (cofeeding) and subsequent cohorts of larvae (Piesman and Spielman 1979, Piesman and Happ 2001, Piesman and Gern 2004). Although the duration of infectivity can vary among strains of *B. burgdorferi*, at least some strains can cause life-long infections in *P. leucopus* populations (Donahue et al. 1987, Lindsay et al. 1997, Derdáková et al. 2004, Hanincová et al. 2008). For these reasons, applying an acaricide to resident mouse populations will likely reduce *I. scapularis* larvae and nymphs infesting those hosts and reduce the efficiency of the *B. burgdorferi* endemic cycle, which ultimately would reduce the DIT.

Both permethrin (topical administration) and fipronil (both topical and oral administration) are acaricidal compounds proven effective to reduce tick infestation of small mammals in the laboratory (e.g., Poché et al. 2020, Poché et al. 2021) and/or in field experiments (e.g., Stafford 1992, Lane et al. 1998, Dolan et al. 2017). Several field studies using these acaricides have shown promising results at reducing the DIT (Mather et al. 1988, Deblinger and Rimmer 1991, Mejlon et al. 1995, Dolan et al. 2004, Schulze et al. 2017); however, other studies reported that such treatments had no impact (Daniels et al. 1991, Stafford 1992, Hinckley et al. 2021).

In 2014, a novel family of systemic acaricides named isoxazolines reached the veterinary drug market (Gassel et al. 2014, Shoop et al. 2014). Drugs of this family (e.g., afoxolaner, fluralaner, sarolaner, and lotilaner) rapidly kill ticks feeding on treated hosts and they continue to do so for weeks, following a single treatment, when used in dogs (Wengenmayer et al. 2014, McTier et al. 2016, Six et al. 2016, Murphy et al. 2017) or cats (Geurden et al. 2017, Cavalleri et al. 2018) against adult *Ixodes* ticks. Afoxolaner and sarolaner kill adult *Ixodes* ticks so rapidly that *B. burgdorferi* transmission does not occur and as a result, treated dogs do not become infected nor do they develop signs of LD (Baker et al. 2016, Honsberger et al. 2016). Isoxazolines have also proven safe when used with a higher frequency of administration than recommended and/or at many times the minimal targeted dose in mammals such as rats (CVMP 2014) and dogs (Walther et al. 2014). Recently fluralaner baits were demonstrated effective in a laboratory setting at reducing the number of *I. scapularis* larvae infesting *Peromycus* mice (Pelletier et al. 2020). However, the laboratory trial showed that the proportion of larvae killed and the duration of this effect in *Peromyscus* mice was lower than reported in dogs, suggesting that repeated treatment of small mammals throughout the tick activity season would be essential to effectively disrupt *B. burgdorferi* transmission.

In this study, we aimed to test the effect of fluralaner baiting on the infestation of wild small mammals by immature *I. scapularis* ticks in a natural environment, as a first step toward the development of an environmental intervention targeting reservoir hosts. Bait stations were used to experimentally deploy fluralaner weekly at two different densities (i.e., 2.1 baits/1,000 m^2 and 4.4 baits/1,000 m^2) over a three-year period (2016–2018) in a well-studied deciduous forest site in Southern Québec, Canada. To assess treatment effectiveness, the number of feeding larvae (FL) and feeding nymphs (FN) on small mammals captured in treated and control plots were compared. We tested the hypothesis that regular deployment of fluralaner baits during the activity period for immature ticks would result in a reduction in the number of ticks per small mammal in treated plots, as killing infesting immature *I. scapularis* represents the first direct effect of reservoir targeted-approach using acaricidal treatment. The effects of isoxazoline treatment on the transmission cycle of *B. burgdorferi*, and consequently on the DIT, will be the object of a separate investigation.

Materiel and Methods

Experimental Site

The experiment took place in a fenced forested area (Fig. 1) located within Farnham National Defense facility where LD cases were reported as early as 2009 (Bourré-Tessier et al. 2011). The site is located in the Estrie region of the province of Québec, Canada, with a reported annual Lyme disease incidence of 12.4–47.5 cases per 100,000 people between 2015 and 2020 (MSSS 2021). The habitat on the site is typical of southern Québec: deciduous forest dominated by maple and oak trees with a thick leaf litter layer and a high density (approx. 14 animals per km^2) of white-tailed deer (Daigle et al. 2004).

The study was conducted during 2016, 2017, and 2018 and study sites were divided into three 350 × 400 m zones including: one control zone (C) and two treatment zones (T1 and T2). Zones C and T1 were established at the start of the study in 2016 and T2 was added in 2017. The three zones were roughly contiguous but separated by

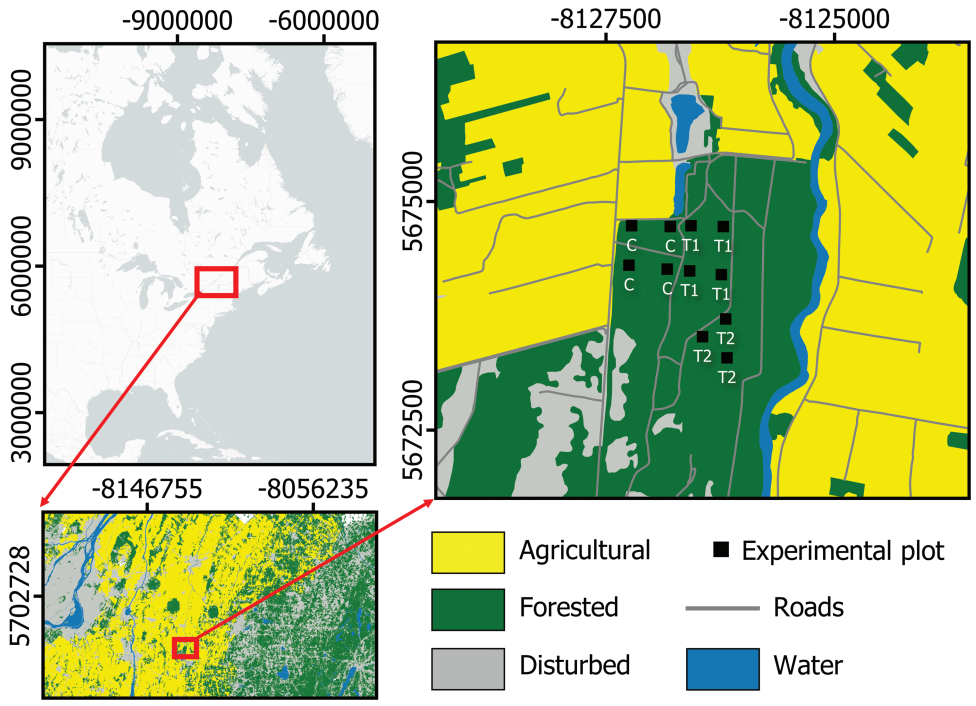

Fig. 1. Location of the study site in northeastern North America and in southern Québec, Canada, and the location of plots within the study site.

at least 250 m or a large gravel access road and drainage ditches, that were expected to limit the mixing of mouse populations between zones (Fig. 1). Each zone contained several 75 × 75m experimental plots where baits were deployed and small mammals were captured. To limit spatial dependence, plot borders were separated by a minimum of 150 m, based on the estimated home range of *Peromyscus* mice (Wolff 1985), such that mice from the individual plots were rarely captured in multiple plots. Zones C and T1 had four plots while Zone T2 was limited to three plots because a large wetland limited accessibility to this area for most of the summer (Fig. 1).

Treatment

The treatment was delivered to small mammals by placing a bait mixture within mouse-size Protecta RTU bait stations (Bell Laboratories Inc. Madison, WI) distributed evenly within each treated plot. The bait was a mixture of peanut butter and the commercial formulation of fluralaner Bravecto (Merck Animal Health, Madison, NJ). Both products were mixed to obtain a mixture with a fluralaner concentration of 4.8 mg per g of bait. Stations were supplied with 250–500 mg of bait mixture containing 1.2–2.4 mg of fluralaner, i.e., enough to provide a dose of 50–100 mg/kg if entirely consumed by a 25 g (range: 12–30 g) *Peromyscus* mouse (Smith et al. 2012). Baits were deployed each summer over a six-week period from July 10th to 15th until August 23rd to 26th to ensure that small mammals had access to the treatment during or before the period of larval activity taking place from the end of July to early September (Ogden et al. 2008). Each week fresh bait was placed into each bait station and the degree of consumption of the previous week's bait was scored as fully consumed, partially consumed, or untouched. Any left-over baits were collected and disposed off site. Zone C received no bait. Plots in zone T1 received a bait density of 2.1 per 1,000 m^2 (12 stations per 5.63 km^2 plot) in 2016, which was increased in 2017 to 4.4 per 1,000 m^2 (25 stations) in 2017 and 2018. Plots in zone T2 received a bait density of 2.1 per 1,000 m^2 in both 2017 and 2018.

Small Mammal Sampling

Small mammals were captured twice each summer in each study plot. Traps were deployed prior to placing baits in the field (i.e., from the last week of June to the first week of July) and near the end of the period that baits were in the field (i.e., during the last week of July in 2016 and in the 3rd and 4th week of August in 2017 and 2018). The second capture period was later in 2017 and 2018 to ensure small mammals were examined at or near the peak period of larval activity.

Small mammals were captured using Sherman live traps (H.B. Sherman Traps, Tallahassee, FL) and 25–35 traps per plot were deployed in a rectangular grid of the same dimensions as the plot (75 × 75 m). Traps were baited with a mixture of peanut butter and oatmeal and supplemented with a slice of apple and water repellent foam to prevent dehydration and improve capture survival, respectively. Traps were activated at 17 h and checked next morning at 6 h. Greater trapping effort was applied in plots where trap success was lower to maximize statistical power. In addition, for animal welfare reasons, captures were not performed when the humidity adjusted heat index exceeded 40°C. See Supp Table 1 [online only] for a complete description of the trapping effort and captures.

Traps containing captured small mammals were taken a short distance (<5 km) to a manipulation station where captured animals were anesthetized using an isoflurane vaporizer. Isoflurane was delivered at a concentration of 5% to render animals unconscious and then it was lowered to 1–2% to maintain anesthesia for a maximum of 20 min per mouse. A subcutaneous injection of a maximum 0.3 ml of 0.9% NaCl was administered to animals that were suspected of being dehydrated. The sex, weight, and species of each animal were recorded, and mice were only identified in the field to the genus level. Each animal was also visually examined for ticks and the number of larvae and nymphs was recorded. Ticks were removed and a subset was identified using keys (INSPQ 2022) to confirm the species infesting small mammals on our study plots. At

first capture, each animal was marked with a unique subcutaneous pit tag (Mini HPT8, Biomark Inc, Boise, ID) and all captured animals were released at the exact location of collection. Any animals recaptured during a sampling period were also released at the site of collection but without processing.

Density of Questing Ticks

Host-seeking ticks were collected in each plot once per small mammal capture period using drag sampling: dragging a 1-m^2 piece of white flannel cloth across the forest floor (Rulison et al. 2013). Tick drags were examined for ticks every 25 m on marked transects and any ticks found were preserved in 70% ethanol for further testing, and identification to life stage and species. In 2016 and 2017, two transects of 75 m within each plot were sampled by period of capture for a total sampling surface of 300 m^2 by year. In 2018, three transects of 75 m by plot were added to increase the total sampling surface by year to 750 m^2.

Statistical Analysis

Study Sample. Only *Peromyscus* spp. mice were included in our analyses because they are the main reservoir host for *B. burgdorferi* in the study region and the primary target of our approach to reduce the DIT in the environment (Donahue et al. 1987, LoGiudice et al. 2003). Statistical analyses were performed on a dataset that included only the first capture of individual mice in each sampling period. Moreover, to avoid potential type I errors caused by data dependency and repeated measures on some animals, animals recaptured between trapping periods were excluded (Clarke 2008).

Outcome Variables and Treatment Exposure. Treatment effect was measured on the number of FL or FN per mouse. These two variables were both used as outcome variables in a generalized linear model (GLM) with a negative binomial distribution to account for overdispersion. Exposure to treatment was modeled as a 3-level factor (0, 2.1, and 4.4 baits per 1,000 m^2). This variable took the value of 2.1 or 4.4 for treated plots during the capture periods coinciding with the deployment of baits. Treatment exposure had a value of zero during the pretreatment capture period for all plots.

Covariates and Random Factors. Variables tested as potential covariates for the number of ticks infesting mice in the dataset were *sex* (male or female), *age* (juvenile, subadult, or adult), *season* (June and July or August), *zone* (C, T1, or T2), *year* of capture (2016, 2017, or 2018), and *density of questing ticks* (log density of either host-seeking nymphs or larvae depending on which outcome was being modeled).

Peromyscus mice were assigned to an age category based on the following weights: <13 g = juvenile, 13–16 g = subadult, and ≥17 g = adult (Martell 1983, Linzey 1989). Age of mice has been shown to influence the number of ticks infesting hosts (Bouchard et al. 2011). Addition of age and sex were tested as an interaction term as past studies suggested that sexually mature males are more often infested than other age–sex population strata (e.g., Tälleklint and Jaenson 1997, Bouchard et al. 2011). *Season* was used for correction to take phenology of ticks into account (Ogden et al. 2008). The inclusion of *zone* as a covariate was explored to control for the possibility that habitat differences among zones might influence local tick population dynamics. To consider data aggregation, dependency, and to control for unmeasured confounding factors, the variable *plot* was tested as a random intercept in mixed GLM (GLMM).

Model Selection. Only covariates with a significant ($P < 0.2$) univariate association with outcome variables were retained for model selection (Dohoo et al. 1997). Associations between each potential covariate were tested with univariate regression to detect collinearity. Variables with an $R^2 > 0.7$ or odds ratios >8 were considered for exclusion due to high potential of collinearity (Dohoo et al. 2014). Both fixed effect and random effect covariate contributions to the model were tested using log-likelihood ratio test (LRT). Manual backward stepwise elimination was used to sequentially drop variables with the highest *P*-value until every covariate had a significant ($P < 0.05$) contribution to the models. Multicollinearity in the final models was tested with the variance inflation factor (VIF ≥ 10) and the model fit was analyzed with Pearson residuals plot and Pearson residual variance test ($P > 0.05$). Spatial autocorrelation was tested with Moran's I test on residuals of the final models ($P > 0.05$).

Infestation Reduction. To analyse the effect of the treatment, the adjusted coefficients (β) of the treatment predictors of the selected models with their confidence intervals were extracted and transformed with the following formula:

$$\text{Reduction}\ (\%) = \left(1 - e^{\beta}\right) * 100$$

Where e^{β} is the ratio of the number of FL or FN per mouse between treated and control plots from each model. In this way, the formula gives the percent reduction of the number of FL or FN on mice attributable to the treatment (Dohoo et al. 2014).

Software. Statistical analyses were performed using R version 4.0.3 (R Core Team 2021) with *lme4* (Bates et al. 2015), *performance* (Lüdecke et al. 2021), *glmmTMB* (Brooks et al. 2017), *DHARMa* (Hartig 2020), and *ggplot2* packages (Wickham 2009).

Ethics Approval

This study was performed in accordance with the regulation of the Canadian Council for Animal Care (CCAC) and the Ministère de l'Agriculture, des Pêcheries et de l'Alimentation du Québec (SEG: 2016SF2052R16, 2017-05-11-2232-16-SF and 2018-3-22-2369-16-S-F). All animal manipulations were performed with the approval of the institutional animal ethics committee of Université de Montréal (16-Rech-1845, 17-Rech-1836 and 18-Rech-1836).

Results

Treatment

In 2017 and 2018, 1,496 baits were administered in zone T1 and T2 corresponding to 1.8g and 3.6g of fluralaner and an overall application density of 14.5 to 29 mg per 1,000 m^2. Of these baits, 1,424 (95%) were fully consumed, 24 (2%) partially consumed and 48 were untouched (3%). The proportion of each consumption category was similar when stratified by zone indicating no difference in consumption between zones.

Small Mammal Sampling

During 2016, 2017, and 2018, a total of 33 nights of sampling were performed and 340 animals were captured (for more details see Supp Table 1 [online only]). In 2016, too few animals were captured in the period before treatment deployment to allow for interpretation of the data and were therefore not analyzed in this study. Based on data from recaptured hosts, only two (0.6%) small mammals switched zones during the study period. Seven (2.1%) animals also switched plots within the same zone. Twenty-eight

animals from three species other than the genus *Peromyscus* were captured (Table 1).

Peromyscus Mice

Of the 312 mice captured, 62% were male and 71% were adults. Fewer mice were captured in 2016 (12%, 37/312) than in 2017 (41%, 128/312) or in 2018 (47%, 147/312). The average number of larvae and nymphs on mice was higher in 2017 than in 2018 or in 2016 (Table 2, Supp Table 2 [online only]). Between the first and second capture periods of each year, the average number of larvae increased and higher larva numbers were observed as expected given tick phenology in the control plots yet decreased in the treated zones. Only zone T2 in 2017 did not follow this pattern (Fig. 2A; Table 2; Supp Table 2 [online only]). However, in that particular capture period and zone, a subgroup of mice (n = 7) had large numbers of infesting larvae (>25) while other mice had very few (≤5 larvae). Conversely, the average number of FN decreased between early and late season capture periods in both control and treated zones (Table 2), an observation confirmed by the distribution of the number of FN per mouse (Fig. 2B).

Model Selection and Effect of the Treatment

Feeding Larvae (FL). The univariate and multivariate analysis did not exclude any variables for model 1 (FL per mouse). Inclusion of the variable *plot* as a random intercept showed nonsignificant contribution to this model (LRT, 0, P = 1). In the final model, the variables *season* and *density of questing larvae* showed a high level of collinearity (VIF ≥ 10). Therefore, two competing models were built: model 1a keeping *season* as covariate and model 1b keeping *density of questing larvae* (Supp Table 3 [online only]). Model 1b was rejected because the Pearson residual test showed a significant residual variance (P = 0.037) suggesting a worse fit compared to model 1a (P = 0.160). Model 1a showed no residual spatial dependency (Moran's I, P = 0.612).

In the selected model (Table 3), bait densities of 2.1 and 4.4/1,000 m^2 were associated with a reduction of 68% (CI95: 51–79%) and 86% (CI95: 77–92%) in the number of larvae feeding on mice (Fig. 3).

Feeding Nymphs (FN). In model 2 (FN per mouse), univariate analysis excluded the variable *zone* (LRT, 0.5, P = 0.918). Variables *year* and *density of host-seeking nymphs* showed high collinearity (R^2 = 0.74).

Table 1. Total number of captured small mammals, and the average number of larvae and nymphs infesting four groups of small mammals

Common name	Taxonomic groups	No. of animals (%)	No. of larvae (% of total)	Average larvae number (range)[a]	No. of nymphs (% of total)	Average nymphs number (range)[a]
White-footed mouse/ deer mouse	*Peromyscus* spp.	312 (92)	2659 (>99)	8.5 (0–64)	344 (>99)	1.1 (0–24)
Red-backed vole	*Myodes gapperi*	25 (7)	12 (<1)	0.5 (0–5)	3 (<1)	0.1 (0–1)
Woodland jumping mouse	*Napaeozapus insignis*	2 (<1)	10 (<1)	5.0 (0–10)	0	0.0
Northern short-tailed shrew	*Blarina bravicauda.*	1 (<1)	1 (<1)	1.0	0	0.0
	Total	340	2,682	7.9 (0–64)	347	1.0 (0–24)

[a]The total number of feeding larvae (FL) or nymphs (FN) divided by the number of animals contributing to the count.

Table 2. Descriptive data of *I. scapularis* tick infestation of *Peromyscus* mice, aggregated by zone and density of baits

Year	Zone	Bait density[a]	Mice[b]	Males/n[c] (%)	Adults/n[c] (%)	No of larvae[d]	Average larva number (range)[e]	QL[e]	No of nymphs[f]	Average nymph number (range)[e]	QN[f]
2016	C	0	19	15/19 (79)	5/18 (28)	74	3.9 (0–7)	5.0	16	0.8 (0–4)	4.8
	T1	2.1	18	7/18 (39)	14/18 (78)	45	2.5 (0–10)	9.8	13	0.7 (0–2)	6.3
2017	C	0	48	24/48 (50)	29/42 (69)	544	11.3 (1–55)	42.8	86	1.6 (0–17)	5.7
	T1	0	28	18/28 (64)	19/28 (68)	497	17.8 (1–55)	24.5	79	2.8 (0–24)	8.3
		4.4	23	16/23 (70)	9/15 (60)	96	4.2 (0–20)	43.7	4	0.2 (0–1)	5.0
	T2	0	13	7/12 (58)	8/12 (67)	172	13.2 (3–55)	27.3	24	1.8 (0–10)	12.9
		2.1	16	9/14 (64)	7/9 (78)	284	17.8 (0–64)	63.5	17	1.1 (0–6)	6.0
2018	C	0	47	26/47 (55)	41/47 (87)	381	8.1 (0–50)	28.9	30	0.6 (0–5)	2.4
	T1	0	28	12/28 (43)	17/26 (65)	185	6.6 (0–18)	12.2	29	1.0 (0–5)	4.0
		4.4	24	13/24 (54)	16/24 (67)	60	2.5 (0–13)	112.8	2	0.1 (0–1)	1.8
	T2	0	23	12/23 (52)	20/23 (87)	234	10.2 (1–27)	22.2	40	1.7 (0–8)	2.9
		2.1	25	13/25 (52)	20/25 (80)	87	3.2 (0–23)	261.8	4	0.2 (0–1)	2.6
Total			312	172/309 (62)	205/287 (71)	2659	8.5 (0–64)	52.6	344	1.1 (0–24)	4.9

[a]Density of baits per 1,000 m^2.
[b]Number of individual mice captured.
[c]Number of animals for which the value of the variables is known.
[d]Total number of feeding larvae (FL) or nymphs (FN) infesting *Peromyscus* mice.
[e]The total number of feeding larvae (FL) or nymphs (FN) divided by the number of mice captured.
[f]The mean density per 100 m^2 of host-seeking larvae (QL) or nymph (QN).

Therefore, two competing models were built: model 2a keeping *year* of capture as covariate and model 2b keeping *density of host-seeking nymphs* (Supp Table 3 [online only]). In both models, both the interaction term between *age* and *sex* (2a: LRT, 3.0, P = 0.223 and 2b: 3.2, P = 0.197) and *age* (2a: 3.2, P = 0.201 and 2b: 4.0, P = 0.137) were dropped. Inclusion of *plot* as a random intercept resulted in no significant improvement in either model (LRT, 0, P = 1). Both final models did not show moderate or high multicollinearity (VIF ≥ 5). Pearson residual tests for model 2a (P = 0.319) and 2b (P = 0.238) were not significant. Model 2a was retained as the final model based on mathematical criteria. It presented a smaller residual variance than model 2b (Supp Table 3 [online only]). Model 2a (Morans's I, P = 0.207) showed no residual spatial autocorrelation.

In this model (Table 4), only the 4.4 bait density showed a significant negative association with the number of nymphs per mouse, resulting in a reduction of 72% (CI95: 22–90%; Fig. 3).

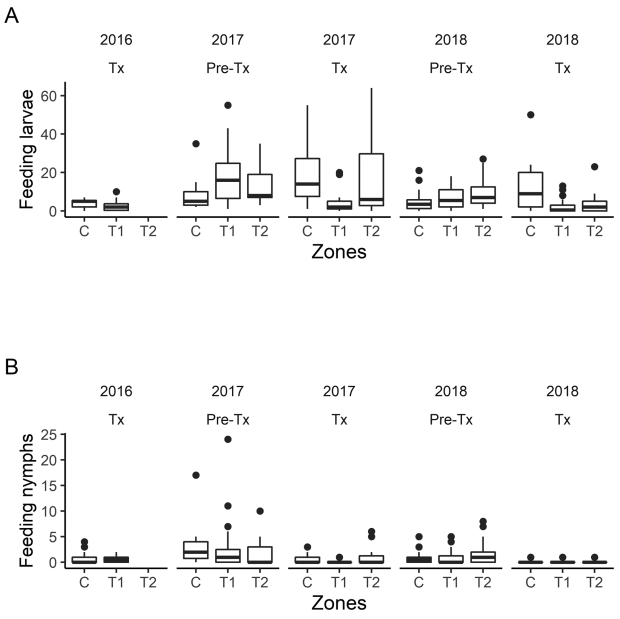

Fig. 2. Boxplots of the number of feeding larvae (A) and feeding nymphs (B) per captured *Peromyscus* mouse by zone, year, and period of capture. Pre-Tx: period of capture before deployment of baits. Tx: period of capture during deployment of baits. Black dots: outliers.

Discussion

In this 3-year trial, fluralaner baits deployed in a natural environment decreased the number of immature *I. scapularis* ticks infesting *Peromyscus* mice. These results demonstrate that oral baiting with fluralaner can kill a significant proportion (up to 86% of FL and 72% of FN at the highest bait density) of immature *I. scapularis* ticks feeding on a key reservoir host of *B. burgdorferi* in northeastern North America (Levine et al. 1985, Mather et al. 1989, Rand et al. 1993, Bouchard et al. 2011). Therefore, results support the hypothesis that a treatment like the one used in this study has the potential to reduce the DIT, particularly in areas where the *B. burgdorferi* endemic cycle is mainly driven by *Peromyscus* mice.

Treatment outcomes varied with bait density, highlighting the impact of treatment strategy on efficacy at reducing tick burdens and, ultimately, on decreasing the DIT. We suspect that the proportion of animals that are treated is an important factor that could influence the association between the treatment and tick infestation of mice. In this study, the difference in effectiveness between the 4.4 and 2.1 baits/1,000 m^2 could be explained by a different proportion of treated mice and/or by different amounts of acaricide administered to mice that consumed baits. These hypotheses were not tested in the current study. Our objective was to measure the effect of different densities of baits in the field on the number of *I. scapularis* larvae or nymphs infesting the resident population of small mammals. All mice captured in active treatment zones were therefore classified as treated rather than attempting to measure the treatment status of individual mice. Future studies should investigate relationships between the treatment strategy, including bait deployment, and small mammal population dynamics. In this regard, it could be important for the design of large-scale interventions to characterize the treatment distribution among small mammal populations, and the association between spatial distribution of baits and the proportion of effectively treated small mammals. The potential positive effect of large-scale interventions on small mammal populations is also worth investigating since bait deployment, which increases local food availability, could potentially increase small mammal population density and thus the DIT (Ostfeld et al. 2006, Gaff et al. 2020). The development of baits without nutritional value (Richer et al. 2014, Stafford et al. 2020) also represents an interesting avenue since it would reduce the potential effect of bait deployment on small mammal populations.

In 2004, Dolan et al deployed up to 1,770 bait boxes treated with a fipronil cotton wick over three years between May and

Table 3. Negative binomial regression model of the number of feeding larvae per mouse (model 1a)

Variables (references)	Categories	β (SE)[a]	P value	CI95[b]
Bait density (0)	2.1	−1.14 (0.22)	<0.001	−1.57; −0.71
	4.4	−1.98 (0.27)	<0.001	−2.50; −1.46
Season (June and July)	August	0.73 (0.16)	<0.001	0.42; 1.04
Year (2016)	2017	1.21 (0.20)	<0.001	0.82; 1.61
	2018	0.54 (0.20)	0.007	0.14; 0.93
Zones (C)	T1	0.52 (0.15)	<0.001	0.24; 0.81
	T2	0.62 (0.16)	<0.001	0.30; 0.94
Sex (Female)	Male	0.59 (0.12)	<0.001	0.36; 0.82
Age (Adults)	Juvenile	0.79 (0.20)	<0.001	0.39; 1.19
	Subadult	0.18 (0.21)	0.395	−0.23; 0.60
Sex*Age (Female*Adult)	Male*Juvenile	−1.06 (0.33)	0.001	−1.70; −0.42
	Male*Subadult	−0.23 (0.26)	0.356	−0.74; 0.27

[a]Estimate regression coefficients with standard error.
[b]95% confidence interval of estimate regression coefficients.

September; and they reported a monthly reduction of the mean number of immature *I. scapularis* ticks on mice between 45 and 96% during application of the treatment. Schulze et al (2017) deployed 78 units of the same device on 10 properties from mid-May to July in 2012 and 2013. They showed a reduction of mean ticks per captured animal between 81 and 100% in treated plots depending on the immature tick stage and the year of observation (Schulze et al. 2017). Studies using topical permethrin tubes also showed similar results (Deblinger and Rimmer 1991, Mejlon et al. 1995). Deblinger and Rimmer (1991) deployed 0.05 tubes/1,000 m^2 from 1987 to 1989. They administrated permethrin to small mammal population once in 1987 and twice in 1988 and 1989, and showed a 100% reduction of larval and nymphal abundance on *P. leucopus* mice (Deblinger and Rimmer 1991). These studies all demonstrated an effect of the treatment of small mammals on the DIT. However, other studies using similar approaches showed a reduction of the infestation of small mammals but no observable effect on the density of host-seeking ticks (Stafford 1992, Daniels et al. 1991). For example, Daniels et al. (1991) deployed 100 permethrin-treated devices on sites in different landscape settings once in August 1987 and twice (May & July) in 1988, resulting in a decreased proportion of infested *P. leucopus* mice, yet no significant effect on the DIT. However, results between studies investigating different reservoir targeted approaches remain difficult to compare because of differences in acaricide deployment protocols, mechanisms of action (systemic for fluralaner vs contact toxicity for permethrin and fipronil), ecological context, and statistical analyses.

In this study, we aimed to quantify the temporal effect of fluralaner treatment by having a control period prior bait deployment in each plot. This design provides a longitudinal baseline for treated plots in addition to the comparison with control plots. In 2016, pre-treatment infestation data were not available due to unusually low trapping success. This may have resulted in an underestimation of treatment effectiveness, particularly in terms of FN per mouse. It is unlikely that, at the scale of our study, the local abundance of questing larvae was strongly impacted by the treatment because of dispersal of engorged female from adjacent untreated landscapes by other hosts such as white-tailed deer (Stafford 1992). However, cohorts of nymphs are highly influenced by cohorts of larvae from the previous year (Dumas et al. 2022). Larval cohorts on which the treatment may have had an effect in 2016 and in 2017 should therefore lower densities of host-seeking nymphs the next year. Another limitation is related to the observation that FL and FN do not necessary fall off the host immediately when they are killed (Pelletier et al 2020). In the present study, all feeding ticks were included in the number of ticks per mouse regardless whether they appeared alive on dead, which may have resulted in an underestimation of the treatment effect.

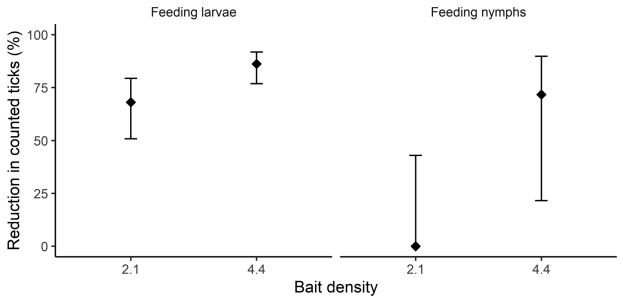

Fig. 3. Percent reduction of larvae and nymphs infesting *Peromyscus* mice (±1 CI95). The variable *bait density* (per 1,000 m^2) is the predictor of the effect of the treatment. Coefficients of the models were used to calculate the proportion of reduction of ticks on mice. In model 1a (FL), coefficients were adjusted for mouse *age* and *sex*, and for *season*, *year*, and *zone* of capture. In model 2a (FN), they were adjusted for the mouse *sex*, *season*, and *zone* of capture.

There are many reasons why killing ticks infesting small mammals may not translate into a reduction of the DIT (Eisen 2021, Hinckley et al. 2021). Ecological determinants of the *B. burgdorferi* endemic cycle are likely to modulate the effect of reservoir-targeted approaches. In particular, host community composition may influence treatment effectiveness in several ways (Tsao et al. 2004, Eisen and Dolan 2016, Eisen 2021). First, species other than *Peromyscus* mice such as shrews, ground foraging birds, and chipmunks are competent reservoir-hosts and contribute to the maintenance of *B. burgdorferi* (Slajchert et al. 1997, LoGiudice et al. 2003, Reed et al. 2003, Ginsberg et al. 2005, Brisson et al. 2008). The baits used in our study are likely less attractive to fossorial insectivore like shrews, and the design of the stations excluded chipmunks and other species that were too large to enter (although future studies could include these species by using larger bait stations like SELECT TCS bait boxes, Tick Box Technology Corporation, Norwalk, CT). Tsao et al (2004), in their study targeting *Peromyscus* mice with a *B. burgdorferi* vaccine, observed that their treatment was less effective at specific sites, and that nymphs at those sites carried different *B. burgdorferi* strains than the ones that commonly infect *Peromyscus* mice. This observation supports the notion that other reservoir species are involved in *B. burgdorferi* endemic cycles (Tsao et al. 2004). In addition, the contribution of white-tailed deer and other hosts of adult ticks, to tick reproduction and population growth may also mitigate the effect of reservoir-targeted interventions on the DIT. High white-tailed deer densities are associated with high *I. scapularis* tick densities in central and eastern North America (Wilson et al. 1985, Rand et al. 2003, Bouchard et al. 2013). They could contribute to increased density of host-seeking ticks in treated zones by

Table 4. Negative binomial regression model of the number of feeding nymphs per mouse (model 2a)

Variables (references)	Categories	β (SE)[a]	*P* value	CI95[b]
Bait density (0)	2.1	0.09 (0.33)	0.794	−0.56; 0.73
	4.4	−1.26 (0.52)	0.015	−2.28; −0.24
Season (June and July)	August	−1.51 (0.29)	<0.001	−2.08; −0.93
Year (2016)	2017	−0.22 (0.35)	0.521	−0.90; 0.46
	2018	−1.06 (0.36)	0.003	−1.76; −0.36
Sex (Female)	Male	0.51 (0.19)	0.007	0.14; 0.88

[a]Estimate regression coefficients with standard error.
[b]95% confidence interval of estimate regression coefficients.

dispersing engorged adult female ticks from untreated zones, with each female tick giving rise to thousands of host-seeking larvae (Piesman and Gern 2004). By adding ticks in the environment, they increase the probability of immature ticks feeding upon infected untreated small mammals and could mitigate the effect of the treatment on the DIT. White-tailed deer can also contribute to feeding a proportion of larvae and nymphs thus preventing them from being exposed to the treatment (Goethert et al. 2021).

In conclusion, this study showed that the administration of an isoxazoline treatment to small mammals in a natural environment using bait stations effectively reduced infestation of a key wildlife reservoir of *B. burgdorferi* in nature by *I. scapularis* larvae and nymphs. This study represents a first step in the development of a reservoir-targeted approach using isoxazolines that aimed at disrupting the *B. burgdorferi* endemic cycle, reducing the DIT in the environment, and preventing transmission of *B. burgdorferi* to humans or pets. This approach has the potential to offer a promising new tool for LD prevention and its use could serve as a component of an integrated vector management strategy provided that isoxazolines are licensed for use in wildlife. In this regard, in addition to confirming the effectiveness at reducing the DIT, more investigations of reservoir-targeted approach using isoxazolines are needed to determine their cost-efficacy, the broader environmental impacts of the treatment (e.g., safety for mice and predators eating treated prey), and potential development of acaricide resistance following repeated treatment.

Acknowledgments

We thank Université de Montréal, Institut national de santé publique du Québec and Natural Sciences and Engineering Research Council of Canada, discovery grant to P.L. (03793-2014), which funded this study. We addressed our deep gratitude to Robert Werbiski and Franck Siriex, from the Canadian Armed Forces, for their collaboration in making this study possible. We also thank all field assistants who participated in this study; this work would not have been possible without their efforts.

Author Contributions

J.P., J.-P.R., C.A., N.H.O., L.R.L., C.B. and P.A.L. participated in the study design and protocol. J.P. and G.D.M. conducted the field trial. J.P. conducted statistical analysis and wrote the manuscript. All authors revised the manuscript. All authors read and approved the final manuscript.

References Cited

Aenishaenslin, C., K. Charland, N. Bower, E. Perez-Trejo, G. Baron, F. Milord, and C. Bouchard. **2022**. Behavioral risk factors associated with tick exposure in a Lyme disease high incidence region in Canada. *BMC Public Health*. 22: 807.

Aenishaenslin, C., J. Pelletier, L. Potes, J.-P. Rocheleau, C. Bouchard, and P. Leighton. **2021**. *Développement et évaluation d'une intervention Une seule santé pour réduire le risque de maladie de Lyme à Bromont - Rapport final. Project report*. Université de Montréal, Montréal.

Baker, C. F., J. W. McCall, S. D. McCall, M. D. Drag, E. B. Mitchell, S. T. Chester, and D. Larsen. **2016**. Ability of an oral formulation of afoxolaner to protect dogs from *Borrelia burgdorferi* infection transmitted by wild *Ixodes scapularis* ticks. *Comp. Immunol. Microbiol. Infect. Dis.* 49: 65–69.

Bates, D., M. Maechler, B. Bolker, and S. Walker. **2015**. Fitting linear mixed-effects models using lme4. *J. Stat. Softw.* 67: 1–48.

Berry, K., J. Bayham, S. R. Meyer, and E. P. Fenichel. **2018**. The allocation of time and risk of Lyme: a case of ecosystem service income and substitution effects. *Environ. Resour. Econ.* 70: 631–650.

Bouchard, C., G. Beauchamp, S. Nguon, L. Trudel, F. Milord, L. R. Lindsay, D. Bélanger, and N. H. Ogden. **2011**. Associations between *Ixodes scapularis* ticks and small mammal hosts in a newly endemic zone in southeastern Canada: implications for *Borrelia burgdorferi* transmission. *Ticks Tick Borne Dis.* 2: 183–190.

Bouchard, C., P. A. Leighton, G. Beauchamp, S. Nguon, L. Trudel, F. Milord, L. R. Lindsay, D. Belanger, and N. H. Ogden. **2013**. Harvested white-tailed deer as sentinel hosts for early establishing *Ixodes scapularis* populations and risk from vector-borne zoonoses in southeastern Canada. *J. Med. Entomol.* 50: 384–393.

Bouchard, C., E. Leonard, J. K. Koffi, Y. Pelcat, A. Peregrine, N. Chilton, K. Rochon, T. Lysyk, L. R. Lindsay, and N. H. Ogden. **2015**. The increasing risk of Lyme disease in Canada. *Can. Vet. J.* 56: 693–699.

Bourré-Tessier, J., F. Milord, C. Pineau, and E. Vinet. **2011**. Indigenous Lyme disease in Quebec. *J. Rheumatol.* 38: 183.

Brisson, D., D. E. Dykhuizen, and R. S. Ostfeld. **2008**. Conspicuous impacts of inconspicuous hosts on the Lyme disease epidemic. *Proc. Biol. Sci.* 275: 227–235.

Brooks, M. E., K. Kristensen, K. J. van Benthem, A. Magnusson, C. W. Berg, A. Nielsen, H. J. Skaug, M. Maechler, and B. M. Bolker. **2017**. glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. *R. J.* 9: 400.

Cavalleri, D., M. Murphy, W. Seewald, J. Drake, and S. Nanchen. **2018**. Laboratory evaluation of the efficacy and speed of kill of lotilaner (Credelio™) against *Ixodes ricinus* ticks on cats. *Parasit. Vectors*. 11: 413.

Clarke, P. **2008**. When can group level clustering be ignored? Multilevel models versus single-level models with sparse data. *J. Epidemiol. Community Health* 62: 752–758.

Committee for Medicinal Products for Veterinary Use (CVMP). **2014**. CVMP assessment report for Bravecto (EMEA/V/C/002526/0000), pp. 22. European Medicine Agency, Amsterdam. https://www.ema.europa.eu/en/documents/assessment-report/bravecto-epar-public-assessment-report_en.pdf.

Daigle, C., M. Crête, L. Lesage, J. -P. Ouellet, and J. Huot. **2004**. Summer diet of two white-tailed deer, Odocoileus virginianus, populations living at low and high density in southern Québec. *Can. Field Nat.* 118: 360–367.

Daniels, T. J., D. Fish, and R. C. Falco. **1991**. Evaluation of host-targeted acaricide for reducing risk of Lyme disease in southern New York state. *J. Med. Entomol.* 28: 537–543.

Deblinger, R. D., and D. W. Rimmer. **1991**. Efficacy of a permethrin-based acaricide to reduce the abundance of *Ixodes dammini* (Acari: Ixodidae). *J. Med. Entomol.* 28: 708–711.

Derdáková, M., V. Dudiòák, B. Brei, J. S. Brownstein, I. Schwartz, and D. Fish. **2004**. Interaction and transmission of two Borrelia burgdorferi sensu stricto strains in a tick-rodent maintenance system. *Appl. Environ. Microbiol.* 70: 6783–6788.

Dohoo, I. R., C. Ducrot, C. Fourichon, A. Donald, and D. Hurnik. **1997**. An overview of techniques for dealing with large numbers of independent variables in epidemiologic studies. *Prev. Vet. Med.* 29: 221–239.

Dohoo, I. R., W. Martin, and H. Stryhn. **2014**. *Veterinary epidemiologic research*, 2nd ed. VER Inc., Charlottetown (PEI), Canada.

Dolan, M. C., G. O. Maupin, B. S. Schneider, C. Denatale, N. Hamon, C. Cole, N. S. Zeidner, and K. C. Stafford, 3rd. **2004**. Control of immature *Ixodes scapularis* (Acari: Ixodidae) on rodent reservoirs of *Borrelia burgdorferi* in a residential community of southeastern Connecticut. *J. Med. Entomol.* 41: 1043–1054.

Dolan, M. C., T. L. Schulze, R. A. Jordan, C. J. Schulze, A. J. Ullmann, A. Hojgaard, M. A. Williams, and J. Piesman. **2017**. Evaluation of doxycycline-laden oral bait and topical fipronil delivered in a single bait box to control *Ixodes scapularis* (Acari: Ixodidae) and reduce *Borrelia burgdorferi* and *Anaplasma phagocytophilum* infection in small mammal reservoirs and host-seeking ticks. *J. Med. Entomol.* 54: 403–410.

Donahue, J. G., J. Piesman, and A. Spielman. **1987**. Reservoir competence of white-footed mice for Lyme disease spirochetes. *Am. J. Trop. Med. Hyg.* 36: 92–96.

Dumas, A., C. Bouchard, L. R. Lindsay, N. H. Ogden, and P. A. Leighton. **2022**. Fine-scale determinants of the spatiotemporal distribution of Ixodes scapularis in Quebec (Canada). *Ticks Tick Borne Dis*. 13: 101833.

Eisen, L. **2021**. Control of ixodid ticks and prevention of tick-borne diseases in the United States: the prospect of a new Lyme disease vaccine and the continuing problem with tick exposure on residential properties. *Ticks Tick Borne Dis*. 12: 1877–9603.

Eisen, L., and M. C. Dolan. **2016**. Evidence for personal protective measures to reduce human contact with blacklegged ticks and for environmentally based control methods to suppress host-seeking blacklegged ticks and reduce infection with Lyme disease spirochetes in tick vectors and rodent reservoirs. *J. Med. Entomol*. 53: 1063–1092.

Eisen, R. J., and L. Eisen. **2018**. The blacklegged tick, *Ixodes scapularis*: an increasing public health concern. *Trends Parasitol*. 34: 295–309.

Eisen, R. J., J. Piesman, E. Zielinski-Gutierrez, and L. Eisen. **2012**. What do we need to know about disease ecology to prevent Lyme disease in the northeastern United States? *J. Med. Entomol*. 49: 11–22.

Fischhoff, I. R., S. E. Bowden, F. Keesing, and R. S. Ostfeld. **2019**. Systematic review and meta-analysis of tick-borne disease risk factors in residential yards, neighborhoods, and beyond. *BMC Infect. Dis*. 19: 861.

Gaff, H., R. J. Eisen, L. Eisen, R. Nadolny, J. Bjork, and A. J. Monaghan. **2020**. LYMESIM 2.0: an updated simulation of blacklegged tick (Acari: Ixodidae) population dynamics and enzootic transmission of *Borrelia burgdorferi* (Spirochaetales: Spirochaetaceae). *J. Med. Entomol*. 57: 715–727.

Gassel, M., C. Wolf, S. Noack, H. Williams, and T. Ilg. **2014**. The novel isoxazoline ectoparasiticide fluralaner: selective inhibition of arthropod gamma-aminobutyric acid- and L-glutamate-gated chloride channels and insecticidal/acaricidal activity. *Insect. Mol. Biol*. 45: 111–124.

Geurden, T., S. Borowski, M. Wozniakiewicz, V. King, J. Fourie, and J. Liebenberg. **2017**. Comparative efficacy of a new spot-on combination product containing selamectin and sarolaner (Stronghold™ plus) versus fluralaner (Bravecto™) against induced infestations with *Ixodes ricinus* ticks on cats. *Parasit. Vectors*. 10: 319.

Ginsberg, H. S., P. A. Buckley, M. G. Balmforth, E. Zhioua, S. Mitra, and F. G. Buckley. **2005**. Reservoir competence of native North American birds for the Lyme disease spirochete, *Borrelia burgdorfieri*. *J. Med. Entomol*. 42: 445–449.

Goethert, H. K., T. N. Mather, J. Buchthal, and S. R. Telford, 3rd. **2021**. Retrotransposon-based blood meal analysis of nymphal deer ticks demonstrates spatiotemporal diversity of Borrelia burgdorferi and Babesia microti reservoirs. *Appl. Environ. Microbiol*. 87(2): e02370-20.

Hanincová, K., N. H. Ogden, M. Diuk-Wasser, C. J. Pappas, R. Iyer, D. Fish, I. Schwartz, and K. Kurtenbach. **2008**. Fitness variation of Borrelia burgdorferi sensu stricto strains in mice. *Appl. Environ. Microbiol*. 74: 153–157.

Hartig, F. **2020**. DHARMa: residual diagnostics for hierarchical (multi-Level/ mixed) regression models. https://cran.r-project.org/web/packages/DHARMa/index.html

Hinckley, A. F., S. A. Niesobecki, N. P. Connally, S. A. Hook, B. J. Biggerstaff, K. A. Horiuchi, A. Hojgaard, P. S. Mead, and J. I. Meek. **2021**. Prevention of Lyme and other tick-borne diseases using a rodent-targeted approach: a randomized controlled trial in Connecticut. *Zoonoses Public Health*. 68: 578–587.

Honsberger, N. A., R. H. Six, T. J. Heinz, A. Weber, S. P. Mahabir, and T. C. Berg. **2016**. Efficacy of sarolaner in the prevention of *Borrelia burgdorferi* and *Anaplasma phagocytophilum* transmission from infected Ixodes scapularis to dogs. *Vet. Parasitol*. 222: 67–72.

Institut Nationale de Santé Publique du Québec (INSPQ). **2022**. Identification guide for ticks found in Québec. Government of Québec. https://www.inspq.qc.ca/en/identification-guide-ticks-found-quebec

Kitron, U., and J. J. Kazmierczak. **1997**. Spatial analysis of the distribution of Lyme disease in Wisconsin. *Am. J. Epidemiol*. 145: 558–566.

Lane, R. S., L. E. Casher, C. A. Peavey, and J. Piesman. **1998**. A better tick-control trap: Modified bait tube controls disease-carrying ticks and fleas. *Hilgardia*. 52: 43–47.

Lane, R. S., S. A. Manweiler, H. A. Stubbs, E. T. Lennette, J. E. Madigan, and P. E. Lavoie. **1992**. Risk factors for Lyme disease in a small rural community in northern California. *Am. J. Epidemiol*. 136: 1358–1368.

Leighton, P. A., J. K. Koffi, Y. Pelcat, L. R. Lindsay, and N. H. Ogden. **2012**. Predicting the speed of tick invasion: an empirical model of range expansion for the Lyme disease vector *Ixodes scapularis* in Canada. *J. Appl. Ecol*. 49: 457–464.

Levine, J. F., M. L. Wilson, and A. Spielman. **1985**. Mice as reservoirs of the Lyme disease spirochete. *Am. J. Epidemiol*. 34: 355–360.

Ley, C., E. M. Olshen, and A. L. Reingold. **1995**. Case-control study of risk factors for incident Lyme disease in California. *Am. J. Epidemiol*. 142: S39–S47.

Lindsay, L. R., I. K. Baker, G. A. Surgeoner, S. A. McEwen, and G. D. Campbell. **1997**. Duration of *Borrelia burgdorferi* infectivity in white-footed mice for the tick vector *Ixodes scapularis* under laboratory and field conditions in Ontario. *J. Wildl. Dis*. 33(4): 766–775.

Linzey, A. V. **1989**. Response of the white-footed mouse (*Peromyscus leucopus*) to the transition between disturbed and undisturbed habitats. *Can. J. Zool*. 67: 505–512.

LoGiudice, K., R. S. Ostfeld, K. A. Schmidt, and F. Keesing. **2003**. The ecology of infectious disease: effects of host diversity and community composition on Lyme disease risk. *PNAS*. 100: 567–571.

Lüdecke, D., M. S. Ben-Shachar, I. Patil, P. Waggoner, and D. Makowski. **2021**. performance: An R Package for assessment, comparison and testing of statistical models. *J. Open Source Softw*. 60: 3139.

Mac, S., S. R. da Silva, and B. Sander. **2019**. The economic burden of Lyme disease and the cost-effectiveness of Lyme disease interventions: a scoping review. *PLoS One*. 14: e0210280.

Mac, S., G. A. Evans, S. N. Patel, E. M. Pullenayegum, and B. Sander. **2021**. Estimating the population health burden of Lyme disease in Ontario, Canada: a microsimulation modelling approach. *CMAJ* 9(4): e1005–e1012.

Martell, A. M. **1983**. Demography of southern red-backed voles (*Clethrionomys gapperi*) and deer mice (*Peromyscus maniculatus*) after logging in north-central Ontario. *Can. J. Zool*. 61: 958–969.

Mather, T. N., J. M. C. Ribeiro, S. I. Moore, and A. Spielmam. **1988**. Reducing transmission of Lyme disease spirochetes in a suburban setting. *Ann. N. Y. Acad. Sci*. 539: 402–403.

Mather, T. N., M. L. Wilson, S. I. Moore, J. M. Ribeiro, and A. Spielman. **1989**. Comparing the relative potential of rodents as reservoirs of the Lyme disease spirochete (*Borrelia burgdorferi*). *Am. J. Epidemiol*. 130: 143–150.

McTier, T. L., R. H. Six, J. J. Fourie, A. Pullins, L. Hedges, S. P. Mahabir, and M. R. Myers. **2016**. Determination of the effective dose of a novel oral formulation of sarolaner (Simparica™) for the treatment and month-long control of fleas and ticks on dogs. *Vet. Parasitol*. 222: 12–17.

Mead, P., S. Hook, S. Niesobecki, J. Ray, J. Meek, M. Delorey, C. Prue, and A. Hinckley. **2018**. Risk factors for tick exposure in suburban settings in the Northeastern United States. *Ticks Tick Borne Dis*. 9: 319–324.

Mejlon, H. A., T. G. Jaenson, and T. N. Mather. **1995**. Evaluation of host-targeted applications of permethrin for control of *Borrelia*-infected *Ixodes ricinus* (Acari: Ixodidae). *Med. Vet. Entomol*. 9: 207–210.

Ministère de la Santé et des Services Sociaux (MSSS). **2021**. Maladie de Lyme: Tableau des cas humains - Archives 2014 à 2020. Governement of Québec, Québec. https://www.msss.gouv.qc.ca/professionnels/zoonoses/maladie-lyme/tableau-des-cas-humains-lyme-archives/

Murphy, M., R. Garcia, D. Karadzovska, D. Cavalleri, D. Snyder, W. Seewald, T. Real, J. Drake, S. Wiseman, and S. Nanchen. **2017**. Laboratory evaluations of the immediate and sustained efficacy of lotilaner (Credelio™) against four common species of ticks affecting dogs in North America. *Parasit. Vectors*. 10: 523.

Ogden, N. H., M. Bigras-Poulin, K. Hanincová, A. Maarouf, C. J. O'Callaghan, and K. Kurtenbach. **2008**. Projected effects of climate change on tick phenology and fitness of pathogens transmitted by the North American tick *Ixodes scapularis*. *J. Theor. Biol*. 254: 621–632.

Ogden, N. H., L. R. Lindsay, M. Morshed, P. N. Sockett, and H. Artsob. **2009**. The emergence of Lyme disease in Canada. *CMAJ Can. Med. Assoc. J*. 180: 1221–1224.

Ostfeld, R. S., A. Price, V. L. Hornbostel, M. A. Benjamin, and F. Keesing. **2006**. Controlling ticks and tick-borne zoonoses with biological and chemical agents. *BioScience*. 56: 383–394.

Patrican, L. A. **1997**. Absence of Lyme disease spirochetes in larval progeny of naturally infected *Ixodes scapularis* (Acari:Ixodidae) fed on dogs. *J. Med. Entomol*. 34: 52–55.

Pelletier, J., J. -P. Rocheleau, C. Aenishaenslin, F. Beaudry, G. Dimitri Masson, L. R. Lindsay, N. H. Ogden, C. Bouchard, and P. A. Leighton. **2020**. Evaluation of fluralaner as an oral acaricide to reduce tick infestation in a wild rodent reservoir of Lyme disease. *Parasit. Vectors*. 13: 73.

Pepin, K. M., R. J. Eisen, P. S. Mead, J. Piesman, D. Fish, A. G. Hoen, A. G. Barbour, S. Hamer, and M. A. Diuk-Wasser. **2012**. Geographic variation in the relationship between human Lyme disease incidence and density of infected host-seeking *Ixodes scapularis* nymphs in the Eastern United States. *Am. J. Trop. Med. Hyg*. 86: 1062–1071.

Piesman, J., and L. Gern. **2004**. Lyme borreliosis in Europe and North America. *Parasitology*. 1: S191–S220.

Piesman, J., and C. M. Happ. **2001**. The efficacy of co-feeding as a means of maintaining *Borrelia burgdorferi*: a North American model system. *J. Vector Ecol*. 26: 216–220.

Piesman, J., and A. Spielman. **1979**. Host-associations and seasonal abundance of immature *Ixodes dammini* in southeastern Massachusetts. *Ann. Entomol. Soc. Am*. 72: 829–832.

Poché, D. M., G. Franckowiak, T. Clarke, B. Tseveenjav, L. Polyakova, and R. M. Poché. **2020**. Efficacy of a low dose fipronil bait against blacklegged tick (*Ixodes scapularis*) larvae feeding on white-footed mice (*Peromyscus leucopus*) under laboratory conditions. *Parasit. Vectors*. 13: 391.

Poché, D. M., K. Dawson, B. Tseveenjav, and R. M. Poché. **2021**. Efficacy of low-dose fipronil bait against blacklegged tick (*Ixodes scapularis*) larvae feeding on white-footed mice (*Peromyscus leucopus*) under simulated field conditions. *Parasit. Vectors*. 14: 459.

Rand, P. W., E. H. Lacombe, R. P. Smith, Jr, S. M. Rich, C. W. Kilpatrick, C. A. Dragoni, and D. Caporale. **1993**. Competence of *Peromyscus maniculatus* (Rodentia: Cricetidae) as a reservoir host for *Borrelia burgdorferi* (Spirochaetares: Spirochaetaceae) in the wild. *J. Med. Entomol*. 30: 614–618.

Rand, P. W., C. Lubelczyk, G. R. Lavigne, S. Elias, M. S. Holman, E. H. Lacombe, and R. P. J. Smith. **2003**. Deer density and the abundance of *Ixodes scapularis* (Acari: Ixodidae). *J. Med. Entomol*. 40: 179–184.

R Core Teams. **2021**. *R: a language and environment for statistical computing*, R Foundation for Statistical, Vienna.

Reed, K. D., J. K. Meece, J. S. Henkel, and S. K. Shukla. **2003**. Birds, migration and emerging zoonoses: West Nile virus, Lyme disease, influenza A and enteropathogens. *Clin. Med. Res*. 1: 5–12.

Richer, L. M., D. Brisson, R. Melo, R. S. Ostfeld, N. Zeidner, and M. Gomes-Solecki. **2014**. Reservoir targeted vaccine against *Borrelia burgdorferi*: a new strategy to prevent Lyme disease transmission. *J. Infect. Dis*. 209: 1972–1980.

Rulison, E. L., I. Kuczaj, G. Pang, G. J. Hickling, J. I. Tsao, and H. S. Ginsberg. **2013**. Flagging versus dragging as sampling methods for nymphal *Ixodes scapularis* (Acari: Ixodidae). *J. Vector Ecol*. 38: 163–167.

Schulze, T. L., R. A. Jordan, M. Williams, and M. C. Dolan. **2017**. Evaluation of the SELECT tick control system (TCS), a host-targeted bait box, to reduce exposure to *Ixodes scapularis* (Acari: Ixodidae) in a Lyme disease endemic area of New Jersey. *J. Med. Entomol*. 54: 1019–1024.

Scoles, G. A., M. Papero, L. Beati, and D. Fish. **2001**. A relapsing fever group spirochete transmitted by *Ixodes scapularis* ticks. *Vector Borne Zoonotic Dis*. 1: 21–34.

Shoop, W. L., E. J. Hartline, B. R. Gould, M. E. Waddell, R. G. McDowell, J. B. Kinney, G. P. Lahm, J. K. Long, M. Xu, T. Wagerle, *et al*. **2014**. Discovery and mode of action of afoxolaner, a new isoxazoline parasiticide for dogs. *Vet. Parasitol*. 201: 179–189.

Six, R. H., D. R. Young, M. R. Myers, and S. P. Mahabir. **2016**. Comparative speed of kill of sarolaner (Simparica) and afoxolaner (NexGard) against induced infestations of Ixodes scapularis on dogs. *Parasit. Vectors*. 9: 79.

Slajchert, T., U. D. Kitron, C. J. Jones, and A. Mannelli. **1997**. Role of the eastern chipmunk (*Tamias striatus*) in the epizootiology of Lyme borreliosis in northwestern Illinois, USA. *J. Wildl. Dis*. 33: 40–46.

Smith, P. F., J. L. Benach, D. J. White, D. F. Stroup, and D. L. Morse. **1988**. Occupational risk of Lyme disease in endemic areas of New York state. *Ann. N. Y. Acad. Sci*. 539: 289–301.

Smith, P. C., J. A. Carlson Scholz, and S. R. Wilson. **2012**. Deer mice, white-footed mice, and their relatives, pp. 1075–1088. *In* M. A. Suckow, K. A. Stevens, and R. P. Wilson (eds.), *The laboratory rabbit, guinea pig, hamster, and other rodents*. Academic Press, Cambridge, MA, USA.

Stafford, K. C., 3rd. **1992**. Third-year evaluation of host-targeted permethrin for the control of *Ixodes dammini* (Acari: Ixodidae) in southeastern Connecticut. *J. Med. Entomol*. 29: 717–720.

Stafford, K. C., 3rd, S. C. Williams, J. G. van Oosterwijk, M. A. Linske, S. Zatechka, L. M. Richer, G. Molaei, C. Przybyszewski, and S. K. Wikel. **2020**. Field evaluation of a novel oral reservoir-targeted vaccine against *Borrelia burgdorferi* utilizing an inactivated whole-cell bacterial antigen expression vehicle. *Exp. Appl. Acarol*. 80: 257–268.

Tälleklint, L., and T. G. Jaenson. **1997**. Infestation of mammals by *Ixodes ricinus* ticks (Acari: Ixodidae) in south-central Sweden. *Exp. Appl. Acarol*. 12: 755–551.

Tsao, J. I., J. T. Wootton, J. Bunikis, M. G. Luna, D. Fish, and A. G. Barbour. **2004**. An ecological approach to preventing human infection: vaccinating wild mouse reservoirs intervenes in the Lyme disease cycle. *PNAS*. 101: 18159–18164.

Vandekerckhove, O., E. De Buck, and E. Van Wijngaerden. **2019**. Lyme disease in Western Europe: an emerging problem? A systematic review. *Acta Clin. Belg*. 76(3): 244–252.

Walther, F. M., M. J. Allan, R. K. Roepke, and M. C. Nuernberger. **2014**. Safety of fluralaner chewable tablets (Bravecto), a novel systemic antiparasitic drug, in dogs after oral administration. *Parasit. Vectors*. 7: 87.

Wengenmayer, C., H. Williams, E. Zschiesche, A. Moritz, J. Langenstein, R. K. A. Roepke, and A. R. Heckeroth. **2014**. The speed of kill of fluralaner (Bravecto™) against Ixodes ricinus ticks on dogs. *Parasit. Vectors*. 7: 525.

Wickham, H. **2009**. ggplot2: Elegant graphics for data analysis. Springer-Verlag, New York.

Wilson, M. L., G. H. Adler, and A. Spielman. **1985**. Correlation between abundance of deer and that of the deer tick, *Ixodes dammini* (Acari: Ixodidae). *Ann. Entomol. Soc. Am*. 78: 172–176.

Wolff, J. O. **1985**. The effects of density, food, and interspecific interference on home range size in *Peromyscus leucopus* and *Peromyscus maniculatus*. *Can. J. Zool*. 63: 2657–2662.

Zhang, X., M. I. Meltzer, C. A. Peña, A. B. Hopkins, L. Wroth, and A. D. Fix. **2006**. Economic impact of Lyme disease. *Emerg. Infect. Dis*. 12: 653–660.