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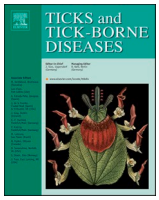

Original article

A rodent and tick bait for controlling white-footed mice (*Peromyscus leucopus*) and blacklegged ticks (*Ixodes scapularis*), the respective pathogen host and vector of the Lyme disease spirochetes.

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ABSTRACT

A promising alternative approach to conventional vector and rodent control practices is the use of a bait containing a rodenticide and acaricide in controlling vectors and pathogen reservoirs concurrently. In the United States, Lyme disease continues to be the most prevalent vector-borne disease with approximately 500,000 Lyme disease cases estimated each year. Previous research has demonstrated the usefulness of a low dose fipronil bait in controlling *Ixodes scapularis* larvae feeding on white-footed mice. However, considering white-footed mice can be an unwanted species because of their association with tick-borne disease and hantaviruses, a combination rodent and tick bait (RTB) might provide a useful alternative to encourage additional community participation in integrated tick management (ITM) efforts. The purpose of this research was to evaluate the use of RTB (0.025 % warfarin, 0.005 % fipronil) in controlling white-footed mice and *I. scapularis* larvae. Studies were designed in part based on Environmental Protection Agency (EPA) guidelines. A laboratory choice test was conducted to evaluate the use of RTB in controlling white-footed mice over 15-day exposure when they were exposed to an alternative diet. Mice were observed every day for mortality and signs of warfarin toxicity. A simulated field test was conducted to evaluate the use of RTB, presented in the presence of an alternative diet, in controlling *I. scapularis* parasitizing white-footed mice over 4-day exposure. Mice were fitted with capsules and manually infested with *I. scapularis* larvae. The inside of each capsule was observed to evaluate tick attachment. Replete larvae detaching from each mouse were collected. Blood was collected from all treatment group mice via cardiac puncture to determine the fipronil sulfone concentration in plasma for each animal. Results indicated that RTB would be adequately consumed in the presence of an alternative diet under laboratory and simulated field conditions. Treatment with RTB resulted in 100 % mortality of white-footed mice during 15-day exposure and prevented 100 % larvae from feeding to repletion during 4-day exposure. All mice succumbing to RTB showed signs of warfarin toxicity. All mice parasitized with ticks that were exposed to RTB had fipronil sulfone detectable in plasma, with even the lowest concentration detected (8.1 parts per billion) controlling 100 % parasitizing *I. scapularis* larvae. The results suggest that RTB could be a useful means of rodent and tick control for use in ITM programs.

1. Introduction

Lyme disease is the most prevalent vector-borne disease in the United States (US) with over 500,000 human cases estimated to occur annually (Kugeler et al., 2015; K.J. 2021). In the Midwestern and Northeastern US, the blacklegged tick (*Ixodes scapularis*) is the vector of the Lyme disease spirochetes (primarily *Borrelia burgdorferi* sensu stricto, and less frequently *Borrelia mayonii*) and the white-footed mouse (*Peromyscus leucopus*), a species of least concern abundant throughout its home range (UICN, 2016), is the primary reservoir host (Wang et al., 2014). The lifecycle of *I. scapularis* takes approximately two years to complete and is composed of four life stages (egg, larva, nymph, adult). After the eggs hatch, the ticks require a blood meal at each subsequent life stage to survive and develop (United States Centers for Disease Control and Prevention, 2020). The risk of human exposure to the causative agents of Lyme disease is a function of the local abundance of nymph and adult ticks (Stafford et al., 1998), being strongly correlated with the density of spirochete-infected ticks in the areas surrounding residences (Mather, 1993). Nymphal tick bites are suggested to be responsible for nearly 90 % of Lyme disease cases each year (Fish, 1993). One potential means of

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preventing human exposure is to target white-footed mice with a systemic acaricide bait to eliminate parasitizing larval *I. scapularis* during blood feeding, subsequently reducing abundance of infected nymphs the following spring.

Oral systemic acaricides have emerged as a promising means of tick control (Eisen, 2023) and an array of compounds have been evaluated for use in controlling fleas and ticks parasitizing small rodents (Borchert and Poché, 2011). Compounds such as fipronil (Poché et al., 2020a, 2021, 2023; Williams et al., 2023) and fluralaner (Pelletier et al., 2020, 2022) have been explicitly evaluated for control of *I. scapularis* under laboratory, semi-field and field conditions. Fipronil is a broad-spectrum insecticide (Raymond-Delpech et al., 2005). When presented orally to a host species, it has demonstrated efficacy in systemically controlling a number of blood feeding arthropods, including fleas (Poché et al., 2017a, 2020b; Eads et al., 2019), phlebotomine sand flies (Ingenloff et al., 2013; Mascari et al., 2013; Poché et al., 2013), mosquitoes (Poché et al., 2015, 2017b), and ticks (Poché et al., 2020a; 2021, 2023; Williams et al., 2023). Recently, an acaricide rodent bait (0.005 % fipronil) was developed and efficacy was evaluated against larval *I. scapularis* parasitizing laboratory-reared white-footed mice. In a no-choice study, when mice were presented fipronil bait for 48 h, 100 % efficacy was obtained when *I. scapularis* were allowed to parasitize mice up to 15 days post-exposure (Poché et al., 2020a). In a simulated field study, 100 % efficacy was obtainable when fipronil was present in plasma at $\geq$8.8 parts per billion (ppb) and up to 35 days post-exposure (D.M. Poché et al., 2021).

One of the major challenges of tick-borne disease prevention is that *I. scapularis* control remains the responsibility of individual homeowners who are tasked with determining how much they want to invest financially, the frequency in which they want to engage in control, and the specific control methods they wish to implement (Eisen and Eisen, 2018; Eisen and Stafford, 2021). While the above studies were useful in demonstrating the effectiveness of a fipronil-based bait in controlling *I. scapularis* parasitizing a critical reservoir host of *B. burgdorferi* s.s, additional supplemental measures should be included in integrated tick management (ITM) programs to encourage greater community participation. Although white-footed mice spend much of their time in fields, when in close proximity with humans they can become an unwanted nuisance. White-footed mice, in addition to serving as reservoirs for vector-borne pathogens, are responsible for human transmission of zoonotic disease agents, such as hantavirus, through urine, droppings and saliva (Luong et al., 2011; Berl et al., 2018; Munir et al., 2021; Valera et al., 2022). White-footed mice may additionally cause economic, structural and agricultural damage, and often move into buildings and make use of and spoil improperly stored food (Whitmer and Moulton, 2012; Witmer, 2022; Diagne et al., 2023). Thus, the impact that white-footed mice pose at the community level and to individual homeowners can transcend the risk of tick-borne diseases. An acaricide-only tick control product may not be appealing to some homeowners who consider the mice adjacent to their homes to be pests and would prefer to remove them rather than feed them non-toxic bait. However, eliminating the rodents without incorporating a method of vector control may cause partially engorged, infected immature ticks to detach from the deceased host and disperse into the surrounding environment in pursuit of an alternative host and complete blood feeding, increasing the risk of vector-borne pathogen transmission in humans and animals (Tahir et al., 2020). A possible solution to both issues would be the application of a combination rodenticide and acaricide product, allowing participating homeowners to control unwanted rodents on their properties while simultaneously participating in the vector control process.

Warfarin, a first-generation anticoagulant used medicinally in humans to treat thrombosis at low concentrations (Link, 1959), was approved by the Food and Drug Administration (FDA) for medicinal use in 1954 (United States Food and Drug Administration, 2011) and by the EPA for rodenticidal use in 1952 (United States Environmental Protection Agency, 1991). When used to control rats and mice, a latency period of several days occurs prior to symptom onset (Hone and Mulligan, 1982; Eason and Ogilvie, 2009), which decreases the likelihood of bait shyness, encouraging continued consumption. The average days until death of laboratory-bred house mice *(Mus musculus*) and Norway rats (*Rattus norvegicus*) exposed to warfarin bait (0.025 %) are 5 and 6 days, respectively, which are similar to that of more toxic second-generation anticoagulants (Poché and Poché, 2012). Warfarin is metabolized quickly, reducing the risk of secondary exposure to scavengers and predators (Cowled and O'Connor, 2004; Poché et al., 2019) and has an antidote, Vitamin K1 (Link, 1959; Thijssen, 1995).

The latency period of warfarin could prove advantageous if incorporated into a combination bait containing low dose fipronil for vector control. *Ixodes scapularis* larvae blood feeding on mice require approximately 4 days to reach full engorgement and detach (Kocan et al., 2015). Unlike acute toxicants such as bromethalin in which symptoms often manifest within 24 h (Dorman, 1990), low dose warfarin requires multiple days for symptoms to occur, which should allow time for fipronil to remove all blood feeding ectoparasites prior to rodent death (Fig. 1).

The purpose of the following studies was to determine the rodenticidal and insecticidal efficacy of a rodent and tick combination bait containing 0.025 % warfarin and 0.005 % fipronil (herein referred to as RTB) against *P. leucopus* mice and *I. scapularis* tick larvae, respectively. Results provide insights into the possible use of a combination bait for controlling *I. scapularis* parasitizing wild white-footed mice to potentially be included in ITM programs.

2. Materials and methods

2.1. White-footed mice and ethics declaration

To conduct these studies, we utilized mice from an in-house outbred *P. leucopus* colony located at Genesis Laboratories, Inc. All procedures performed with animals and all study protocols were in accordance with United States Department of Agriculture Animal Welfare Act regulations and the National Research Council's Guide for Care and Use of Laboratory Animals and were approved by the Genesis Institutional Animal Care and Use Committee (Genesis Laboratories Protocols 19,006, 20,002).

2.2. Rodent and tick bait preparation

Two large batches of RTB were produced. Raw ingredients were blended in a large industry standard mixer and extruded in a wax block formulation. The formulation was similar to the fipronil wax block utilized by Poché et al. (2020a, 2021), containing fipronil at a nominal concentration of 0.005 % (50 parts per million (ppm)). However, the present formulation also contained 0.025 % (250 ppm) warfarin, which is the nominal concentration recommended by EPA for warfarin-based rodenticide products (United States Environmental Protection Agency, 1991a). A sample of the RTB was analyzed for warfarin and fipronil concentration using a validated high-performance liquid chromatography method. The mean active ingredient concentrations ($\pm$SD) within the two batches were 265.8 $\pm$ 4.0 ppm and 261.9 $\pm$ 18.0 (warfarin) and 47.6 $\pm$ 1.8 ppm and 49.7 $\pm$ 6.5 ppm (fipronil).

2.3. Experimental design

2.3.1. Study 1: choice test evaluating warfarin

To evaluate the efficacy of RTB as a rodenticide to control white-footed mice, a study was designed and conducted in accordance with US EPA Office of Pesticide Programs (OPP) guidelines regarding anticoagulant dry baits administered to *Peromyscus* spp. mice (United States Environmental Protection Agency, 1991b). A total of 60 mice (30 males, 30 females) were used. Three groups were utilized (2 Treatment, 1

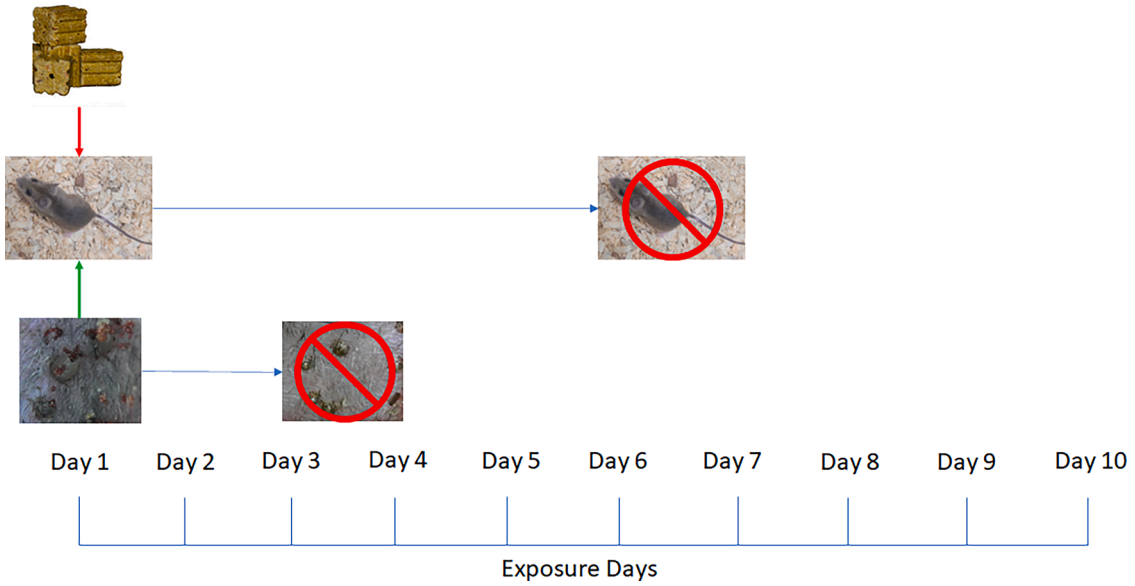

Fig. 1. Conceptualization of a rodent and tick bait containing 0.025 % warfarin and 0.005 % fipronil in *Peromyscus leucopus* and *Ixodes scapularis* control. Mice consume the bait continuously over several days. Ticks are eliminated prior to reaching repletion and detaching, a process which takes approximately 4 days. The average days until death for white-footed mice ranges from 5 to 7 days, suggesting that ticks will be eliminated prior to the mouse being eliminated.

Control), each composed of 20 animals (10 males, 10 females). Mice were housed in groups of five separated by sex. Each group housing was identified by a unique number on the cage and individual mice in each group-housing were identified by unique ear marking. All body weights were recorded to the nearest 0.1 g two days prior to the start of exposure, and at death or at study termination.

Throughout the study duration, mice were housed in groups of five of the same sex in screen bottom metal cages with a surface area of approximately 1536 cm^2. Each cage was raised above absorbent material and contained three polyvinyl chloride (PVC) shelters. Wood shavings positioned under the cages were used as absorbent material and were changed weekly. The photoperiod within the room was maintained at 12 h light: 12 h dark.

RTB was presented to all Treatment group mice alongside an EPA approved *Peromyscus* field rodent challenge diet (United States Environmental Protection Agency, 1991b) in a 15 day choice test. The challenge diet was prepared in the laboratory and was a formulation composed exclusively of pulverized commercial rodent diet and rolled oats. Approximately 50 g RTB and 50 g challenge diet were presented to mice in separate identical stainless-steel feed containers on opposite sides at the front of the cage. The feed containers were spaced so that they were equidistance from the walls, shelters, and the water source and the positions of the two containers were reversed each day to eliminate positional bias. Paper plates were positioned underneath the cages below each feed container to collect spillage. Consumption was measured daily to the nearest 0.1 g.

Mice were observed daily for signs of anticoagulant poisoning. All treatment mice that died during the exposure period were necropsied.

2.3.2. *Study 2: simulated field test evaluating fipronil*

To evaluate the efficacy of RTB as an acaricide in controlling *I. scapularis* larvae parasitizing white-footed mice, a study was designed and implemented in part in accordance with EPA Office of Prevention, Pesticides and Toxic Substances (OPPTS) guidelines (United States Environmental Protection Agency, 1998) and based on a simulated field study design described in D.M. Poché et al. (2021). However, the inclusion of the rodenticide (warfarin) in the formulation, and its potential lethality to mice in the current study, required the experimental design to be modified considerably. Warfarin is an anticoagulant, and it was determined that attaching the capsules to mice during treatment or post-treatment could result in hemorrhaging. Therefore, the capsules were attached to mice during the acclimation period. Additionally, it was decided that during the current study treatment and tick infestation would each occur concurrently rather than the previous method of attaching ticks during a post-treatment period (Poché et al., 2020a, 2021). These modifications resulted in an exposure period of 4 days.

2.3.2.1. Capsule attachment. On Day 0 of acclimation, feeding capsules were attached to each mouse. Each tick feeding capsule was made from a 1.5 ml centrifuge tube using methods described by Poché et al. (2020a). Prior to capsule attachment, mice were individually placed into an induction chamber and anesthetized using an isoflurane vaporizer set to 4 % isoflurane with an oxygen flow rate of 2 L/min. After being anesthetized, each mouse was removed from the induction chamber and attached to a nosecone and the isoflurane dispersal rate and oxygen flow rate were respectively reduced to 2 % and 0.5 L/min. A portion of fur on the back near the shoulder blades was trimmed using an Equine FX™ horse shearer (Conair Corp., Stanford, CT, USA). The capsule was then secured to the skin using a natural adhesive (3 parts rosin:1 part beeswax). Mice were then returned to group housing and monitored until fully recovered from anesthesia. Mice were allowed to acclimate for 3 days.

2.3.2.2. Rodent and tick bait exposure. A total of 40 mice (20 male, 20 female) were utilized. Two Treatment groups and two Control groups were utilized and were each composed of 10 mice (5 female, 5 male). Throughout the study, mice were housed in groups ($n = 5$) in metal stock tanks having a surface area ~11,000 cm^2. Ticks were obtained directly from the Oklahoma State Tick Rearing Facility (Stillwater, OK) and were housed in a custom-made desiccator containing a saturated solution of potassium sulfate (140 g potassium sulfate: 1 L water) to maintain relative humidity at >90 %. Temperature and relative humidity were monitored in the test room and were maintained at approximately 20–25 °C and 30–70 %, respectively.

On Day-0 of exposure, approximately 100 g of RTB was presented to the treatment groups in a single commercial bait station positioned against the wall of each enclosure. Approximately 100 g challenge diet was presented as an alternative food source at the opposing wall of the enclosure in a steel plate. The RTB and challenge diet were equidistant from the water source and shelter. Test group mice were exposed to the

RTB and challenge diet for a total of four days. Control group mice remained untreated receiving challenge diet exclusively. Group consumption was recorded daily.

2.3.2.3. Tick attachment. Concurrently with Day 0 exposure, mice were manually infested with ticks, which were applied in the feeding capsule attached to each mouse. Mice were anesthetized using the previously described procedures. While each mouse was anesthetized and on the nose cone, 40 actively questing larvae were applied to the feeding capsule using a fine tipped paint brush. A lid was applied to the top of the capsule and secured to prevent ticks from escaping through the top of the capsule. At the conclusion of tick attachments, mice were returned to the group enclosures and monitored until fully recovered. The rim of each enclosure was coated with petroleum jelly to ensure that no ticks escaped. As a secondary means of collecting escaped ticks, double-sided tape was placed around the perimeter of the room and at the room entrance.

2.3.2.4. Post-Tick attachment. Similar to Poché et al. (2020a, 2021) the post-tick attachment observation period spanned the 4 days following tick attachment. Unlike these previous studies, bait exposure and post-tick attachment occurred concurrently, and thus mice remained in group enclosures for the majority of the post-tick attachment period. Capsule observations were conducted from Day 3 to Day 4 exposure. For each capsule observation, mice were again anesthetized using the previously described method. Each mouse was then individually placed underneath a microscope so that the inside of the capsule could be clearly observed. The inside of the capsule was then carefully scanned for any observable, attached larvae. Attached larvae were those which were imbedded in the skin of the mice and attempting/having attempted to blood-feed. Larvae within the capsules were identified as "non--engorging" (not blood feeding often deceased and desiccated) and "engorging" (actively feeding bloated and white, red, or pink in color), or "replete" (detached and fully fed). Any mice that succumbed to warfarin toxicity prior to the conclusion of the 4-day post-attachment period were immediately placed under the microscope and the ticks were observed. At the conclusion of weighing consumption on Day 4 exposure, mice were suspended above water in individual cages as previously described in Poché et al. (2020a, 2021, 2023) to allow replete larvae detaching from the mice to be more easily collected and to prevent crowding within the capsules. Mice remained in the cages until approximately 15:00, after which Day 4 capsule observations were performed.

2.3.2.5. Blood collection. At the conclusion of the exposure period all treatment group mice and two control group mice were euthanized for blood sample collection. Mice were first anesthetized in the same manner as described above. While anesthetized, mice were euthanized using cervical dislocation in accordance with recommended procedures (Leary et al., 2020). Approximately 100 μl blood was quickly collected from each animal via cardiac puncture using a 1cc syringe with 28-gauge needle. Blood samples were placed in a centrifuge and spun at 6100 repetitions per minute for 10 min. Each plasma sample was then transferred to a individual 1.5 ml centrifuge tube and each tube was immediately stored at −20 C. Plasma was delivered to the Center for Environmental Medicine (CEM) Analytical Laboratory at Colorado State University (Fort Collins, CO) for analysis of fipronil residues. The fipronil concentrations in plasma (ppb) were analyzed using a validated liquid chromatography-mass spectrometry method (LC-MS) previously utilized (Poché et al. 2023). The limit of quantification for detection was 0.5 ppm.

2.4. Data analyses

2.4.1. White-footed mouse body weights

Body weights of all mice were recorded prior to RTB exposure and at study termination or death. Potential differences in body weight between test groups (treatment vs. control) and within each test group (initial weight vs. final weight) were estimated.

2.4.2. Rodent and tick bait consumption

RTB and challenge diet consumption were calculated daily (to the nearest 0.1 g) for each mouse group. For RTB, the proportion (%) of overall diet consumed by each treatment group (acceptance) was calculated using the following formula:

$$Acceptance\ (\%) = 100 * \left(\frac{RTB}{RTB + CD}\right)$$

Where:
RTB= Total RTB Consumed
CD= Total challenge diet consumed

We then used the total bait consumed in each group to estimate the average total warfarin and fipronil consumed by each mouse each day. The body weights taken prior to fipronil bait exposure were then used to estimate total warfarin and fipronil consumption in mg/kg per individual mouse. Differences in consumption of RTB, relative to challenge diet were estimated within the Treatment groups.

2.4.3. Mouse observations

Mice were observed daily for signs of warfarin toxicity. Mortality was recorded daily and used to develop survivorship curves for male and female mice. These data were used to estimate the average days until death for mice exposed to RTB. Mice succumbing to warfarin toxicity were immediately necropsied.

2.4.4. Tick observations and recovery

At Day-3 and Day-4 exposure, the lids of the capsules were removed and mice were placed under the microscope so that the inside of the capsules could be observed. The purpose was to determine whether larvae were successfully engorging, detaching and feeding to repletion. The status of each attached larva observable within the capsules (non-engorging, engorging) was recorded at Day-3 and Day-4 during the post-tick attachment period. Any detached, replete larvae observed during this time were also collected and recorded. Definitions are listed below:

Attached

Non-engorged= Attached larvae, often expired and desiccated and/or having no discernable blood meal observed within the capsule via microscopy. This category additionally includes larvae previously engorging at Day 3 that were eliminated by RTB by Day 4.

Engorging= attached, actively feeding, bloated larvae (often white, pink, or black in color) observed in the capsule via microscopy.

Detached

Replete= Fully engorged detached larvae collected from within the capsules or when placed over water moats on Day 4. These larvae were placed into test tubes and placed into an industry-standard desiccator.

Differences in (i) bodyweights, (ii) daily consumption, and (iii) the numbers non-engorging, engorging and detached replete larvae per test group were analyzed between two groups (Treatment vs Control) and within each test group (initial vs final value) utilizing a student's *t*-test ($P \leq 0.05$).

2.4.5. Fipronil plasma concentration

The fipronil plasma concentration (ng/ml) was estimated for each individual mouse euthanized ($n = 43$). The limit of quantification was 0.5 ng fipronil/ml plasma. Comparisons were made between the numbers of engorging and replete larvae collected from each mouse, relative to the presence of fipronil in plasma samples.

2.4.6. Mortality estimates

The efficacy of 1) warfarin in controlling white-footed mice, and 2) fipronil in preventing *I. scapularis* from engorging and feeding to repletion, was estimated using the following equation (Abbott, 1925).

$$Efficacy\ (\%) = 100 * \left(\frac{C - T}{C}\right)$$

Where:
T= Treatment Group
C= Control Group

All statistical and data analyses were performed using the current versions of JMP Statistical Software (Version 15) (Cary, NC, USA) and Microsoft Excel.

3. Results

3.1. Body weights

Body weight data for both studies are presented in Table 1. During the choice test evaluating warfarin efficacy, the average initial weights of mice in test groups ranged from 21.9 g to 19.1 g. Average final weights ranged from 23.9 g to 18.5 g. Control group males and females on average gained weight by the conclusion of the study while males and females within the Treatment group on average lost weight. However, no significant differences were detected when comparing Treatment and Control group initial and final body weights or when comparing initial and final body weights within each group. During the simulated field test evaluating fipronil efficacy, average initial weights of mice in test groups ranged from 22.8 g to 18.4 g. Average final weights ranged from 22.9 g to 16.9 g. Male and female mice within the Treatment groups on average lost weight. Final body weights were significantly greater within the Control groups, relative to the Treatment groups (Students t: t=−2.342, p = 0.0256). Within the Treatment groups, initial weights were significantly greater than final weights (Students t: t = 2.179, p = 0.0359). No significant differences were detected when comparing the initial weights of Treatment and Control or when comparing initial and final weights within Control.

3.2. Consumption

During the 15-day choice test, mice within the Treatment groups consumed a total of 290.2 g of RTB and 347.5 g of challenge diet (Table 2). Cumulative RTB acceptance was estimated to be 45.5 %. Male and female mice consumed 2.4 and 1.5 g bait/day, 309.9 mg/kg and 241.3 mg/kg warfarin, and 62 mg/kg and 48.3 mg/kg fipronil, respectively (Table 3). During the 4-day simulated field test, Treatment group mice consumed 110.9 g RTB and 141.2 g challenge diet during exposure (Table 2). Male and female mice consumed 1.5 and 1.3 g bait/day, 71.5 mg/kg and 68.8 mg/kg warfarin and 14.3 mg/kg and 13.8 mg/kg fipronil, respectively (Table 3). Cumulative RTB acceptance was estimated to be 44.0 %. Differences in challenge diet and RTB consumption were not significant in the choice test or simulated field test.

Table 1
Mean initial and final body weights (±Standard deviation) for test group *Peromyscus leucopus* mice during 15 day choice test and 4 day simulated field test.

Study Type (Exposure duration)	Group	Sex	Initial Weight	Final Weight (Death/End of Post-Exposure)
Choice Test (15-day)	Control (n = 20)	Male (n = 10)	21.3 ± 3.4	23.9 ± 3.7
		Female (n = 10)	19.1 ± 2.1	19.7 ± 2.4
	Treatment (n = 40)	Male (n = 20)	21.7 ± 2.8	20.8 ± 2.9
		Female (n = 20)	20.0 ± 3.0	19.1 ± 3.1
Simulated Field Test (4-day)	Control (n = 20)	Male (n = 10)	21.1 ± 3.7	20.6 ± 3.8[#]
		Female (n = 10)	21.1 ± 3.7	19.6 ± 2.5[#]
	Treatment (n = 20)	Male (n = 10)	20.5 ± 1.9[$]	18.9 ± 1.6[#,$]
		Female (n = 10)	19.0 ± 2.9[$]	17.5 ± 2.1[#,$]

[#] Control significantly greater than Treatment (Students t: t=−2.342, p = 0.0256).
[$] Initial Weight significantly greater than Final Weight (Students t: t = 2.179, p = 0.0359).

3.3. Mouse observations and efficacy (Choice-Test)

All mice within the Treatment groups exhibited signs of anticoagulant toxicity, such as internal hemorrhaging and lethargy. The first symptoms of RTB toxicity were observed on Day 2 of exposure. Throughout exposure, adverse effects were observed, including hyporeactivity, hemorrhaging and death with symptom onset typically occurring within 12 h prior to mortality. Control group mice appeared normal and healthy for the entire exposure and post-exposure periods, with no adverse observations being made. The Rodent-Tick Bait resulted in 100 % mortality of treatment group mice by Day 13 of the exposure period, with the first mice being found dead at Day 3 of exposure (Fig. 2). Mortality peaked on Day 7 of exposure (n = 10) and the average days until death was 7.

3.4. Tick observations, efficacy and plasma collection (Simulated field test)

Anticoagulant symptoms were not as prevalent relative to the 15-day choice test (in part resulting from the reduced exposure period) but were present in some mice. A total of 7 mice within the treatment groups showed signs of anticoagulant toxicity by Day 4 exposure, with one being found dead on Day 4.

The average number of non-engorging, engorging, and detached replete larvae observed per mouse at Day 3 and Day 4 exposure are presented in Table 4. There were a significantly greater number of non-engorging (deceased) larvae present on Treatment group mice, relative to untreated Control (Student's t: t = 18.660, p < 0.0001). In contrast, there was a significantly greater number of engorging (Student's t: t=−6.867, p < 0.0001) and replete (Student's t: t=−4.807 p < 0.0001)

Table 2
Total consumption of Rodent and Tick Bait (RTB) and challenge diet (CD) by treatment group *Peromyscus leucopus* mice during 15-day choice test and 4-day simulated field test.

Study Type (Exposure duration)	Sex	Diet	Total Consumption (g)	Acceptance (%)
Choice Test (15-day)	Male (n = 20)	CD	176.3	45.3
		RTB	146	
	Female (n = 20)	CD	171.2	45.7
		RTB	144.2	
	Combined (n = 40)	CD	347.5	45.5
		RTB	290.2	
Simulated Field Test (4-day)	Male (n = 10)	CD	81.0	42.0
		RTB	58.6	
	Female (n = 10)	CD	60.2	46.5
		RTB	52.3	
	Combined (n = 20)	CD	141.2	44.0
		RTB	110.9	

Acceptance calculated using the following equation:

$$Acceptance\ (\%) = 100 * \left(\frac{RTB}{RTB + CD}\right)$$

Table 3
Average consumption of Rodent and Tick Bait (RTB), warfarin, and fipronil per *Peromyscus leucopus* mouse during 15 day choice test and 4 day simulated field test.

Study Type	Sex	Exposure length (Days)	RTB Consumption/ Mouse (g)	RTB Consumption/ Mouse/Day (g)	Warfarin Consumption/ Mouse (mg)	Fipronil Consumption/ Mouse (mg)	Warfarin Consumption/ Mouse (mg/kg)	Fipronil Consumption/ Mouse (mg/kg)
Choice Test (n = 40)	Male (n = 20)	11	26.9	2.4	6.73	1.35	309.91	61.98
	Female (n = 20)	13	19.3	1.5	4.83	0.97	241.25	48.25
Simulated Field Test (n = 20)	Male (n = 10)	4	5.9	1.5	1.47	0.29	71.46	14.29
	Female (n = 10)	4	5.2	1.3	1.31	0.26	68.82	13.76

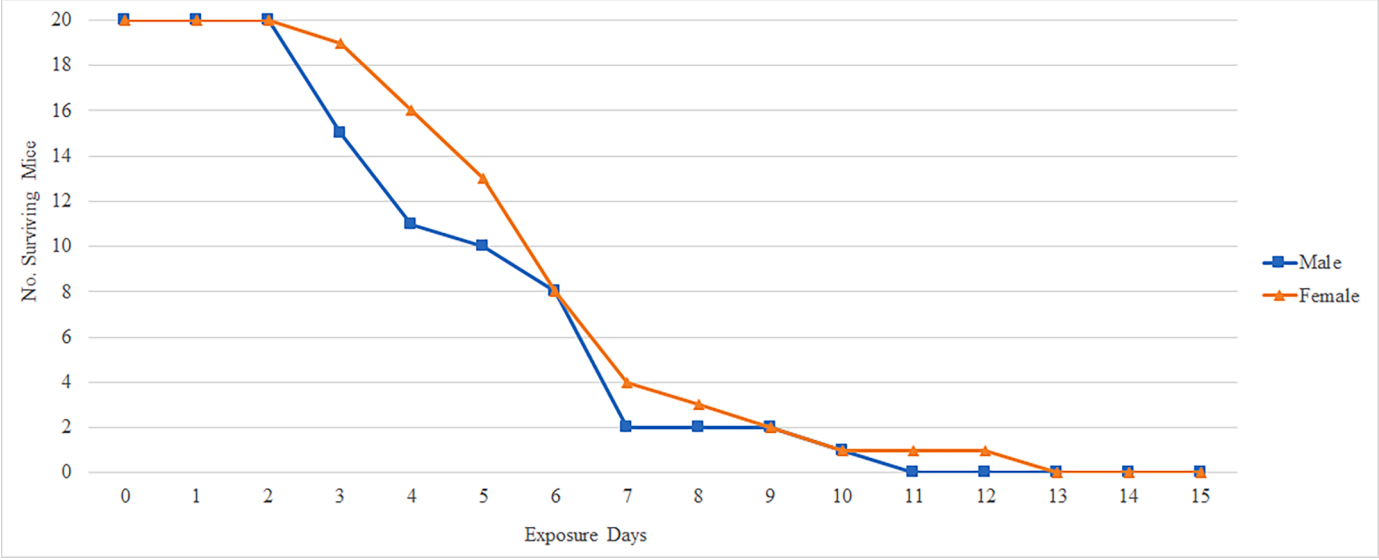

Fig. 2. Survivorship of male and female *Peromyscus leucopus* within the Rodent and Tick Bait treatment groups. One hundred percent (100 %) mortality was obtained for mice within the treatment groups, with the average days until death being 7 days and all mice found dead by Day 13 exposure. No mice died within the Control group.

Table 4
Average (±standard deviation) non-engorging, engorging, replete *Ixodes scapularis* tick larvae per *Peromyscus leucopus* mouse during 4-day simulated field test.

Test Group	Tick Status Post-Tick Attachment					Efficacy in Preventing Repletion (%)
	Day 3		Day 4			
	Non-engorging[#]	Engorging[$]	Non-engorging[#]	Engorging[$]	Replete[&]	
Control 1 (n = 10 mice)	0.3 ± 0.7	13.2 ± 4.0	0.2 ± 0.6	0.6 ± 0.8	14.1 ± 5.7	100
Treatment 1 (n = 10 mice)	8.9 ± 2.1	1.6 ± 3.0	11.5 ± 2.8	0	0	
Control 2 (n = 10 mice)	0.2 ± 0.7	12.9 ± 3.0	0	0.4 ± 0.9	14.5 ± 6.5	100
Treatment 2 (n = 10 mice)	10.7 ± 2.7	1.8 ± 3.0	13.0 ± 3.2	0	0	

[#] Treatment significantly greater than Control (Student's t: $t = 18.660$, $p < 0.0001$).
[$] Control significantly greater than Treatment (Student's t: $t = -6.867$, $p < 0.0001$).
[&] Control significantly greater than Treatment (Student's t: $t = -4.807$ $p < 0.0001$).

larvae on Control group mice relative to Treatment. RTB treatment resulted in 100 % efficacy in preventing larvae from engorging to full repletion and detaching by Day 4 exposure. Every Treatment group mouse that had plasma sampled had fipronil sulfone in plasma above the limit of quantification (0.5 ppb) (Table 5). The average fipronil sulfone in plasma was 1032.0 ± 628.4 ppb with the highest being 2676.8 ppb and the lowest 8.1 ppb. Of 20 mice, 13 had 0 engorging larvae at Day 3 exposure (65 %). Not surprisingly, the individual mouse with 8.1 ppb fipronil sulfone had the most engorging larvae at Day 3 (n = 10). No *I. scapularis* larvae parasitizing mice with fipronil sulfone in plasma >limit of quantification were alive at the conclusion of Day 4, and thus no larvae were able to feed to repletion and detach within the Treatment groups. In contrast, 100 % of mice within the Control groups had larvae which fed to repletion and detached, ranging from 5 to 26 detached replete larvae per mouse.

4. Discussion

The results indicate the potential for RTB containing 0.025 % warfarin and 0.005 % fipronil to effectively control white-footed mice and parasitizing larval *I. scapularis*. Results further suggest the latency period of the anticoagulant provides sufficient time for the *I. scapularis* to succumb to fipronil prior to mouse mortality. Thus, the bait has potential to simultaneously control *I. scapularis* and white-footed mice. The

Table 5
Fipronil sulfone concentrations in *Peromyscus leucopus* mouse plasma, and no. engorging and detached replete *Ixodes scapularis* tick larvae, for each treatment and control mouse during 4 day simulated field test.

Group	Group ID	Sex	Fipronil Sulfone (ppb)	No. Engorging Larvae		Detached Replete Larvae
				Day 3	Day 4	
Treatment 1	T1	F	NA*	0	0	0
Treatment 1	T1	F	952.3	0	0	0
Treatment 1	T1	F	1401.8	0	0	0
Treatment 1	T1	F	479.2	0	0	0
Treatment 1	T1	F	144.6	0	0	0
Treatment 1	T1	M	2020.8	0	0	0
Treatment 1	T1	M	437.9	3	0	0
Treatment 1	T1	M	8.1	10	0	0
Treatment 1	T1	M	828.2	0	0	0
Treatment 1	T1	M	1065.4	3	0	0
Treatment 2	T2	F	1000.0	0	0	0
Treatment 2	T2	F	884.2	0	0	0
Treatment 2	T2	F	1000.0	0	0	0
Treatment 2	T2	F	1514.3	9	0	0
Treatment 2	T2	F	923.1	6	0	0
Treatment 2	T2	M	707.2	0	0	0
Treatment 2	T2	M	1434.4	1	0	0
Treatment 2	T2	M	1386.2	0	0	0
Treatment 2	T2	M	743.1	0	0	0
Treatment 2	T2	M	2676.8	2	0	0
Control 1	C1	F	0.0	18	0	23
Control 1	C1	M	0.0	12	0	11

* NA=Not applicable. Mouse died prior to Day 4 capsule observations and thus plasma could not be collected. All attached larvae observed at Day 3 and Day 4 on this mouse were non-engorging and deceased.

results of these studies led to the federal registration of a product available for rodent and tick control on March 22, 2022 (Kaput® Rodent & Tick Bait: EPA Reg No. 72,500–30).

While mice in the test groups exposed to RTB on average lost weight over the course of the study, the bait remained palatable. During both studies, RTB presented in the presence of an alternative diet exceeded the 33 % acceptance required by EPA for federal registration (United States Environmental Protection Agency, 1991b). The symptomatic latency period of low dose anticoagulant rodenticides encourages continuous feeding over several days, and because symptoms of toxicity are delayed rodents are unable to associate onset of illness with the bait, which prevents bait shyness from occurring (Saxena, 2014). It took multiple days of feeding to succumb to warfarin toxicity, and mice typically did not exhibit symptoms until approximately 12 h prior to mortality, allowing for more than enough bait to be consumed to induce tick mortality prior to the onset of symptoms in mice. This is further evidenced by the concentrations of fipronil sulfone in the plasma of mice.

The efficacy of the rodent and tick bait against the target organism was 100 % during each study. One hundred percent (100 %) mortality of white-footed mice was obtained within 13 days with the average time until death being 7 days. Within the 4-day study, 100 % of *I. scapularis* larvae were prevented from feeding to repletion and detaching. Within the latter study, all Treatment group mice that were sampled for plasma had fipronil sulfone present above the limit of quantification. The lowest concentration obtained (8.1 ppb) resulted in 100 % control of blood feeding *I. scapularis*, which is supported by similar results presented by D.M. Poché et al. (2021) in which 100 % control of *I. scapularis* was obtained when fipronil sulfone in plasma was at or above 8.8 ppb. Additional studies confirming efficacy against *I. scapularis* nymphs could be useful.

Interestingly, while the study evaluating tick control was of a significantly shorter duration, when comparing the rates of mortality at Day 4 of exposure, survivorship of white-footed mice was far greater during the simulated field test relative to the choice test. Only 1 female mouse was found dead within the simulated field test, whereas during the choice test 4 females and 9 males were found dead by the end of Day 4. The daily rate of consumption per mouse in choice test males (2.4 g) was greater, relative to males within the simulated field test (1.5 g), which could explain the accelerated rate of mortality in these males. However, the daily rate of consumption per mouse was similar between female mice within the choice test (1.5 g) and simulated field test (1.3 g), and the results of plasma analysis conducted during the simulated field test indicates that all mice within the Treatment groups were eating a considerable amount of RTB. The mortality rates may have partially been a biproduct of the study designs, and it should be reiterated that the studies were designed to aid in federal registration based on the relevant federal guidelines (EPA, 1991b, 1998). The mortality rate was higher in the choice test where mice were grouped in cages according to OPP guidelines (EPA, 1991b). The smaller surface area, and thus higher density of mice, may have accelerated warfarin toxicity within the choice group. The delayed onset of symptoms within the simulated field test group is encouraging for vector control and indicated that 100 % tick control is obtainable prior to rodent mortality. It is additionally encouraging that the one mouse that died during the exposure period during the simulated field test had consumed enough to eliminate all larvae by Day 3 exposure. This suggests that an exposure rate that eliminates mice within 4 days will also be lethal to ticks. The presence of anticoagulant symptoms at Day 4 in addition to the weight loss observed within Treatment groups suggests that the mortality rate would have increased, and we suspect that 100 % mortality would occur if exposure had been continued. However, in order to be certain, future research might consider evaluating the efficacy of warfarin under simulated field conditions in a 15-day test to confirm that mortality will eventually reach 90–100 % for mice.

Primary rationale for utilizing warfarin is the reduced risk it presents to non-targets, relative to more toxic second-generation anticoagulants. Previous environmental risk assessments have determined warfarin to be moderately toxic to virtually non-toxic to upland game birds and waterfowl (United States Environmental Protection Agency, 1991a). As mentioned previously, warfarin is of reduced secondary risk to scavengers and predators because it is quickly metabolized. However, care should be taken to reduce primary exposure of non-target wildlife to RTB. While RTB is an EPA approved commercial product, the EPA label must be followed to reduce environmental risk. The EPA requires that RTB be applied in tamper-resistant bait stations either indoors or outdoors within 100 ft (~30 m) of buildings. Use of bait stations reduces risk of exposure to children and non-target wildlife. We stress the importance of utilizing a proper bait station, particularly if RTB is to be applied on the outside of buildings. The bait stations should be securely anchored to the ground to prevent removal. If utilizing acaricide-only products to target vertebrate hosts, it is advantageous to utilize a bait station large enough to pass other potential *B. burgdorferi* reservoirs such as *Tamias* spp. chipmunks (McLean et al., 1993). However, for

rodenticidal compounds, limiting access by non-target rodents is important. Thus, managers should utilize a bait station large enough for access by small potentially unwanted rodents listed on the products label, such as *P. leucopus* and *M. musculus* mice, but small enough to restrict access by non-target small rodents such as chipmunks or voles (PROTECTA RTU Mouse Bait Station, Bell Laboratories, Murray Hill, NJ, USA).

No single tick control method used in isolation is likely to adequately reduce *I. scapularis* abundance (Eisen and Gray, 2016) and a number of management practices will need to be integrated to truly have an impact on Lyme disease incidence. Additional studies evaluating an array of integrated tick and pathogen management strategies to control *I. scapularis* and reduce risk of infected tick bites are urgently needed (Eisen and Dolan, 2016). These approaches must consider a number of factors, including efficacy of the proposed approaches and the likelihood of community-level participation. Considering a substantial proportion of *I. scapularis* have been collected from within 1 m of the lawn edge of residential properties in the northeast (Stafford and Magnarelli, 1993) a product targeting rodents and ticks adjacent to properties could be beneficial. A rodent and tick product, used near human residences, could provide a welcome companion piece to oral acaricide products utilized to control ticks on mice and deer, the advantage being the removal of pest rodents which might persuade otherwise unwilling homeowners from taking part in tick management.

4.1. Conclusion

The RTB (Kaput ® Rodent & Tick Bait) is a federally approved commercial product (EPA Reg. 72,500–30) that may provide an alternative means of tick control to be incorporated into ITM programs. Neighborhood-wide use of a fipronil-only or RTB, selected by individual homeowners based on their personal preference, could prove to be an ITM strategy encouraging broader community participation. A field trial would be useful in confirming the use of RTB under natural conditions.

Author statement

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David Poché: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Richard Poché:** Writing – review & editing, Supervision, Resources, Methodology, Funding acquisition, Conceptualization.

Data availability

Data will be made available on request.

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