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Nest box-deployed bait for delivering oral vaccines to white-footed mice

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Abstract

Although a wide range of interventions are available for use in reducing the public health burden of Lyme disease, additional tools are needed. Vaccinating mouse reservoirs may reduce the prevalence of spirochetal infection due to the powerful vector and reservoir competence-modulating effects of anti-outer surface protein A (OspA) antibody. A delivery system for an oral immunogen would be required for field trials of any candidate vaccine. Accordingly, we tested candidate bait preparations that were designed to be environmentally stable, attractive to mice, and non-nutritive. In addition, we determined whether delivery of such baits within nest boxes could effectively target white-footed mice. A peanut butter-scented bait was preferred by mice over a blueberry-scented one. At a deployment rate of 12.5 nest boxes per hectare, more than half of resident mice ingested a rhodamine-containing bait, as demonstrated by fluorescent staining of their vibrissae. We conclude that a peanut butter-scented hardened bait placed within simple wood nest boxes would effectively deliver vaccine to white-footed mice, thereby providing baseline information critical for designing field trials of a candidate oral vaccine.

Keywords

Lyme disease; White-footed mice; *Borrelia burgdorferi*; Bait; Oral vaccination; Deer ticks

Introduction

Despite great improvements in public awareness and the availability of acaricidal modes of intervention, the spirochetal agent of Lyme disease now infects more than 20,000 people in the United States every year (Hadler, 2010). The geographical distribution of Lyme disease appears to be increasing as a result of intense development of habitat for housing or

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recreation and increases in deer density (Brownstein et al., 2005), suggesting that the incidence of this zoonosis may greatly increase over the next decades. Although a variety of interventions at the individual and community level are available, to date risk reduction seems to rarely be undertaken. Deer reduction reduces deer tick (the colloquial name for northern human biting populations of ticks that we refer to as *Ixodes dammini*, the junior subjective synonym of *I. scapularis*) densities in discrete sites over the long term (Telford, 2002), but sociopolitical considerations may limit the use of this effective intervention. Habitat modification on a large scale is impractical given constraints on the use of fire to modify the landscape, or the scarcity of funds to maintain brush reduction. Host-targeted acaricides such as Damminix or '4 posters' (Mather et al., 1987; Hoen et al., 2009) based upon coating fur of a host with permethrin or other chemical by means of nesting cotton or an oil wick proximal to bait reduces the density of all host-seeking stages of deer ticks, but their expense and the fact that they must be deployed indefinitely also deters their widespread use. Ground-based spraying of acaricides dramatically reduce tick densities in residential neighborhoods for weeks at a time (Stafford, 1997), but many communities are averse even to the relatively small amounts of low-risk chemicals that may be used in their environment. Public awareness and education remain our most powerful tools, and have undoubtedly reduced risk in many communities where individuals are motivated to use personal protective measures and seek medical attention promptly. At the public health scale, our best hope is to promote the principles of integrated pest management and continue to seek complementary additional strategies. Additional modes of intervention may help reduce risk. Oral vaccination using a recombinant rabies virus protein in a vaccinia vector has been extensively and safely used to reduce the transmission of rabies within European fox populations and eastern U.S. raccoons. We have previously demonstrated that oral delivery of vaccinia expressing the spirochetal outer surface protein A (OspA) protects mice from Lyme disease spirochetes in the laboratory (Scheckelhoff et al., 2006). Anti-OspA antibody reduces the spirochetal competence of vector ticks (Fikrig et al., 1992) and that of reservoir mice as well (Rosa Brunet et al., 1997). In many northeastern U.S. sites, the white-footed mouse (*Peromyscus leucopus*) serves as the main reservoir for the agent of Lyme disease (Levine et al., 1985). It may be that oral vaccination of such mice would reduce the force of spirochetal transmission by rendering them non-infectious to ticks (Fikrig et al., 1993; Tsao et al., 2004).

Towards this end, we evaluated a non-nutritive bait formulation designed to effectively deliver candidate vaccines to white-footed mice. In particular, we determined whether a blueberry or peanut butter-scented formulation was more attractive. In addition, we tested the candidate bait formulation using a simple delivery strategy over the course of 2 years at a study site. The proportion of mice that ingested bait was determined by in vivo marking with rhodamine B (Fisher et al., 1999). The parameters that we developed for bait delivery will greatly facilitate a field trial of an oral transmission-blocking vaccine targeted to white-footed mice.

Materials and methods

Bait formulations

Bait blocks (15–25 g, 5×5×0.75 cm) designated WFM-PB2 (peanut butter scent) and WFM-BB1 (blueberry scent) were developed and produced at FoodSource Lures (Birmingham, Alabama, USA). In addition, 2 textures were evaluated, one with a smooth, hard surface similar to rubber and another with a rough cookie-like surface. Although the exact formulation is proprietary, the bait blocks comprise a hardened alginate and gelatin matrix that is non-nutritive; baits were similar in hardness and flexibility to layers of duct tape applied to 1 cm thickness. Rhodamine 123 (0.5% weight/volume) was incorporated into WFM-PB2 for field trials designed to measure the proportion of mice in a field site that would ingest such bait.

Cage experiment to determine palatability

To determine whether the texture of the bait formulation influenced its attractiveness, we measured the weight loss for individual blocks of bait presented in either large (rat-sized) or small (standard shoebox) cages of mice. White-footed mice (*P. leucopus fuscus*, 4–5 months old, random sex, MV strain; Tufts University) were held (4 mice per small cage, 6 mice per large cage) with ad libitum access to standard rodent chow and water, and 3 bait preparations (WFM-PB2, WFM-BB1, WFM-PB1, designated A, B, and C, respectively) provided. Each bait was weighed to the nearest gram (Pesola 60 gram, Kapuskasing, Ontario) and simultaneously added to each cage, thus each cage had 3 baits in addition to normal mouse chow; baits were placed next to each other. Baits were weighed at days 0, 2, and 4. The experiment was replicated 3 times, using different mice for each replicate.

Attractiveness of candidate bait formulations

To determine whether peanut butter or fruit (blueberry) flavor/smell was more likely to attract white-footed mice, we baited Longworth live traps (Penlon, Abingdon, UK) with equivalent sized blocks (about 3–5 g each) of WFM-PB2 and WFM-BB1 baits, which have the same texture and density. Half of the traps (n=20) received the former bait formulation and the other half (n=20) the latter; baits were alternated between consecutive traps. Traps were set 5 m apart in line transects over 7 days in early-mid September 2008 on the grounds of the Tufts University School of Veterinary Medicine in Grafton, MA, which is surrounded by 150 ha of a successional deciduous forest. Deer ticks are common in this site, and the agent of Lyme disease is enzootic.

Field trial of the attractiveness of bait block candidate WFM-PB2

To determine whether our bait block candidate would be attractive to a natural population of mice that were resident in a site with natural food sources, we undertook a capture-mark release study from April 2009 to June 2010 on the grounds of the Tufts Veterinary School campus. Two 7×7 trapping grids with trap stations 7.6 m apart (about 0.4 ha, Wilson et al., 1985) were established 1500 m apart in successional deciduous forest. The grids comprised a mature red oak (ca. 30 years) canopy with a poison ivy and greenbrier understory. One oats- and cotton-baited Longworth trap was set at each of the 49 trap stations on each grid

every 3 weeks, weather permitting; we did not trap from December to March due to concerns of mouse mortality related to freezing.

To deliver the bait candidate, 5 nest boxes were deployed on each grid, with one box in each corner and the final one in the center. One Grid 1, a commercially available (Mill Stores, Westboro, MA), inexpensive bluebird box (20×15×15cm) made of unfinished pine was used. On Grid 2, a custom made, larger nest box (25×20×20cm) made of exterior grade hardwood was used (Fig. 1). For both, the only entry comprised a 2.5-cm diameter hole; a spacer was placed adjacent to the entry so that the box could be cable tied to a tree with the entrance facing the tree. Mice could enter and exit from between the tree and the box, but no entrance was visible on the other 3 sides, thereby reducing raccoon disturbance.

A single bait block containing rhodamine was placed within a box; blocks were replaced as they were consumed. To facilitate bait replacement, nest boxes were modified so that the top was hinged. Such bait was continuously available within nest boxes for the duration of the experiment.

Mouse trapping

Mice were live-trapped for 2 nights each trap session. Each received a uniquely numbered monel ear tag (#1005-1, National Band and Tag, Newport, KY) and demographic data recorded (sex, reproductive status, juvenile pelage, weight in grams). A vibrissa was randomly selected and stored in a microcentrifuge tube marked with the mouse eartag number and date of capture. Samples were removed from mice only for their first capture at each trap session.

Assay for rhodamine exposure

Vibrissae were mounted on microscope slides in buffered polyvinyl alcohol (Immunomount, Shandon Inc.), coverslipped, and examined under epifluorescence at ×250. Rhodamine filters (excitation 510 nm, emission 530 nm) were used for epifluorescence. The microscopist had no information on the status of the mouse from which the slide derived other than tag number and date. Vibrissae were scored as negative (no fluorescence), or on a +, ++, +++ scale. In addition, the location of fluorescent bands (distal third, center, or proximal third of the shaft; hair root) was recorded with the assumption that due to hair growth, the presence of multiple bands would reflect multiple exposure to rhodamine.

Statistical analyses

Contingency table analyses were conducted where appropriate using Fisher's exact test with significance set a priori at $P < 0.05$.

Animal use approval

All experiments were performed under protocols approved by the Tufts University IACUC (numbers G520-04; G895-07).

Results

To determine which candidate bait formulation would be ingested even in the presence of mouse chow (ad libitum), a choice of 3 bait blocks was presented to each cage. Mice tended to quickly ingest PB2 (Fig. 2) over the 4 days of exposure. We conclude that PB2, even in the presence of rodent chow ad libitum and in either small or large cages, is more attractive than either BB1 or the smooth formulation of PB.

To determine the palatability of our candidate baits comprising either a peanut butter or blueberry scent, we baited Longworth traps with such bait blocks and compared the trap success. Of 240 trap nights, a total of 22 mice was captured. PB2-baited traps accounted for 68% of the 22 captures and BB1-baited traps 32% of 22 ($P=0.12$, Fisher's exact test). We selected the peanut butter-scented WFM-PB2 bait candidate for further evaluation based on the cage experiments as well as because we captured twice as many mice (even though statistically there was no difference in capture success) in the field test.

We determined the proportion of mice that ingested bait over the course of 11 months within a natural habitat. Bait was deployed in 5 nest boxes within each of 2 0.4-ha trapping grids. A total of 85 mice was trapped and ear-tagged over 1617 trap nights (5.3% success). A median of 6 mice (range, 1–23) per grid were present in our sites at any trapping date. Of these 85 mice, 55% yielded vibrissae containing fluorescent bands (Fig. 3). Assuming that vibrissae grow at a constant rate, 8 mice (17%) were considered to have ingested bait on at least 2 different occasions because fluorescent bands were observed at discrete sites on an individual vibrissa. Fourteen (30%) of the mice that had ingested bait had extensive areas of rhodamine staining on their vibrissae, often a third of the shaft, suggesting that they had fed on the bait repeatedly over the course of consecutive days. Interestingly, only 45% of 40 mice trapped on Grid 1 had ingested bait, whereas 64% of 45 mice had ingested bait on Grid 2 ($P=0.08$, Fisher's exact test), suggesting that the larger-size nest box might be more effective in attracting mice/delivering the bait. We conclude that a simple delivery mode using 12.5 nest boxes per ha with our bait candidate is sufficient to expose half of resident mice in a site to rhodamine-containing baits.

Discussion

An integrated pest management approach is needed to reduce the risk of acquiring Lyme disease, comprising a spectrum from short (e.g., personal protection) to long-term (deer reduction) approaches (Stafford, 2008). The success of the live recombinant vaccinia virus expressing a rabies virus glycoprotein (Brochier et al., 1991) in reducing the spread of rabies among foxes in Europe and raccoons in the eastern U.S. provides the rationale for a similar approach for other zoonoses. Indeed, attempts to develop oral vaccine strategies include those targeting brucellosis in Greater Yellowstone bison (http://www.nrmssc.usgs.gov/projects/BR_vaccine_delivery.htm), anthrax in African ungulates (<http://stinet.dtic.mil/oai/oai>), tuberculosis in New Zealand brushtail possums (Buddle et al., 2006), and Puumala virus in bank voles (Khattak et al., 2004), among others. Because of the powerful transmission-blocking activity of recombinant OspA immunization (Fikrig et al., 1992; Rosa Brunet et al., 1997), oral vaccination of the main mouse reservoirs of the agent of Lyme

disease has been proposed (Tsao et al., 2004; Gomes-Solecki et al., 2006) as a means of reducing the force of spirochetal transmission in nature.

In most northeastern U.S. sites, during most years, white-footed mice are generally considered to be the main reservoirs for the deer tick microbial guild because they are very common, are infested most densely by subadult deer ticks, and appear to be highly infectious to uninfected ticks ('reservoir competent': Levine et al., 1985; Donahue et al., 1987; Telford et al., 1996, 1997). Proof of principle for reducing white-footed mouse reservoir capacity for Lyme disease spirochetes in the field has been reported. A field trial of parenterally vaccinating mouse reservoirs was undertaken with interesting results (Tsao et al., 2004). Of nearly 1000 mice that were captured during the 2-year study, 70% seroconverted to OspA. The prevalence of spirochetal infection in host-seeking nymphal deer ticks during the subsequent transmission seasons was reduced by about 15% relative to the control sites, where mice received a sham inoculation. Interestingly, it was estimated that in the absence of mice (or rendering all mice non-infectious), the prevalence of spirochetal infection in host-seeking nymphal deer ticks would be reduced by 65%. Thus, although other species of animals have varying degrees of reservoir capacity for the agent of Lyme disease, a substantive reduction in risk would be evident by targeting solely mice. Oral delivery of a vaccinia virus expressing OspA protects white-footed mice from tick-transmitted spirochetal infection (Scheckelhoff et al., 2006), and other expression systems used in oral vaccination appear to protect mice in the laboratory (Gomes Salecki et al., 2006). These promising results suggest that an oral vaccination strategy targeted to white-footed mice should be evaluated in field trials as a mode of intervention to complement other modes of integrated tick management while second-generation vaccines are developed to target other species with reservoir capacity.

Peanut butter tends to be a universal attractant for rodents; mammalogists have long used peanut butter as bait for traps (Patric, 1970). Accordingly, it was not surprising that a peanut butter-scented bait was attractive to white-footed mice. However, the amount of peanut butter present in the baits is likely to be non-nutritive so that excess food would not help to increase mouse density. The hardened, low-moisture alginate and gelatin matrix provides a vehicle for vaccine delivery that is environmentally stable, viz., not serve as a substrate for fungal contaminants or insects. In actively used nest boxes, baits tended to remain fungus-free; boxes with wet interiors (due to placement issues or poor construction) did cause bait to deteriorate, but they also were less likely to be occupied by mice. The hardness of the baits prevented arthropods such as ants or isopods from eating them, an issue with the use of peanut butter or most other foodstuffs. Although it is possible that an oral vaccine to reduce reservoir capacity for Lyme disease spirochetes may be deliverable over large areas by airplane, it seems more likely that the use of such a mode of intervention would be limited due to cost or concerns regarding potential human exposure. Bait boxes are frequently used to deliver rodenticides, and permanently deployed wooden nest boxes have been effectively used to study the demography or ectoparasites of white-footed mice (Kesner and Linzey, 1997; Drummond, 1957; Jackson and Defoliart, 1975). Vaccine deployment in nest boxes would reduce exposure to humans, and using the protocol that we tested would preferentially target mice over any other animal. Our peanut butter-scented non-nutritive baits deployed within 12.5 simple wooden nest boxes per ha will expose at least 50% of

mice within a site, and provide a practical deployment strategy for future field trials of oral vaccination of mice to reduce the force of spirochetal transmission.

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References

- Brochier B, Kieny MP, Costy F, Coppens P, Bauduin B, Lecocq JP, Languet B, Chappuis G, Desmettre P, Afiademyo K. Large-scale eradication of rabies using recombinant vaccinia-rabies vaccine. *Nature*. 1991; 354:520–522. [PubMed: 1758494]
- Brownstein JS, Skelly DK, Holford TR, Fish D. Forest fragmentation predicts local scale heterogeneity of Lyme disease risk. *Oecologia*. 2005; 146:469–475. [PubMed: 16187106]
- Buddle BM, Aldwell FE, Keen DL, Parlane NA, Hamel KL, de Lisle GW. Oral vaccination of brushtail possums with BCG: investigation into factors that may influence vaccine efficacy and determination of duration of protection. *N.Z. Vet.J.* 2006; 54:224–230. [PubMed: 17028659]
- Donahue JG, Piesman J, Spielman A. Reservoir competence of white-footed mice for Lyme disease spirochetes. *Am. J. Trop. Med. Hyg.* 1987; 36:92–96. [PubMed: 3812887]
- Drummond RO. Observations on fluctuations of acarine populations from nests of *Peromyscus leucopus*. *Ecological Monographs*. 1957; 27:137–152.
- Fikrig E, Telford SR III, Barthold SW, Kantor F, Spielman A, Flavell RA. Elimination of *Borrelia burgdorferi* from vector ticks feeding on OspA-immunized mice. *Proc. Natl. Acad. Sci. USA*. 1992; 89:5418–5421. [PubMed: 1608951]
- Fisher P. Review of using Rhodamine B as a marker for wildlife studies. *Wildl. Soc. Bull.* 1999; 27:318–329.
- Gomes-Solecki MJ, Brisson DR, Dattwyler RJ. Oral vaccine that breaks the transmission cycle of the Lyme disease spirochete can be delivered via bait. *Vaccine*. 2006; 24:4440–4449. [PubMed: 16198456]
- Hadler, JL. Disease surveillance and case definitions in tick-borne diseases. Institute of Medicine Committee on Lyme Disease and Other Tick-Borne Diseases: The State of the Science. Critical Needs and Gaps in Understanding Prevention, Amelioration, and Resolution of Lyme and Other Tick-Borne Diseases; 2010. p. A226-A246. <http://www.nap.edu/catalog/13134.html>
- Hoen AG, Rollend LG, Papero MA, Carroll JF, Daniels TJ, Mather TN, Schulze TL, Stafford KC III, Fish D. Effects of tick control by acaricide self-treatment of white-tailed deer on host-seeking tick infection prevalence and entomologic risk for *Ixodes scapularis* -borne pathogens. *Vector Borne Zoonotic Dis.* 2009; 9:431–438. [PubMed: 19650738]
- Jackson JO, Defoliart GR. Some quantitative relationships of certain nidicolous acari and the white-footed mouse, *Peromyscus leucopus*. *J. Med. Entomol.* 1975; 12:323–332. [PubMed: 1181437]
- Kesner MH, Linzey AV. Modeling population variation in *Peromyscus leucopus* : an exploratory analysis. *J. Mammal.* 1997; 78:643–654.
- Khattak S, Darai G, Rosen-Wolff DA. Puumala virus nucleocapsid protein expressed in transgenic plants is not immunogenic after oral administration. *Virus Genes*. 2004; 29:109–116. [PubMed: 15215689]
- Levine JF, Wilson ML, Spielman A. Mice as reservoirs of the Lyme disease spirochete. *Am. J. Trop. Med. Hyg.* 1985; 34:355–360. [PubMed: 3985277]
- Mather TN, Ribeiro JMC, Spielman A. Lyme disease and babesiosis: acaricide focused on potentially infected ticks. *Am. J. Trop. Med. Hyg.* 1987; 36:609–614. [PubMed: 3555140]
- Patric E. Bait preference of small mammals. *J. Mammal.* 1970; 51:179–182.
- Rosa Brunet L, Katavolos P, Spielman A, Telford SR III. Anti-OspA reduces reservoir competence of mice for *Borrelia burgdorferi* the agent of Lyme disease. *Med. Vet. Entomol.* 1997; 11:198–200. [PubMed: 9226653]

- Scheckelhoff ML, Telford SR III, Hu L. Protective efficacy of an oral vaccine to reduce carriage of *Borrelia burgdorferi* (strain N40) in mouse and tick reservoirs. *Vaccine*. 2006; 24:1949–1957. [PubMed: 16300863]
- Stafford KC III. Pesticide use by licensed applicators for the control of *Ixodes scapularis* (Acari: Ixodidae) in Connecticut. *J. Med. Entomol.* 1997; 34:552–558. [PubMed: 9379461]
- Stafford, KC, III. Tick Management Handbook. revised edition. New Haven, CT: Connecticut Agricultural Experiment Station Bulletin 1010; 2007. p. 84
- Telford SR III, Dawson JE, Katavalos P, Warner CK, Kolbert CP, Persing DH. Perpetuation of the agent of human granulocytic ehrlichiosis in a deer tick-rodent cycle. *Proc. Nat. Acad. Sci. USA*. 1996; 93:6209–6214. [PubMed: 8650245]
- Telford SR III, Armstrong PM, Katavalos P, Foppa I, Garcia Olmeda AS, Wilson ML, Spielman A. A new tick-borne encephalitis-like virus infecting deer ticks, *Ixodes dammini* *Emerg. Infect. Dis.* 1997; 3:165–170.
- Telford, SR, III. Deer tick-transmitted zoonoses in the eastern United States. Aguirre, A.; Ostfeld, RS.; Tabor, GM.; House, C.; Pearl, MC., editors. Oxford University Press, NY: Conservation Medicine: Ecological Health in Practice; 2002. p. 310-324.
- Tsao JI, Wootton JT, Bunikis J, Luna MG, Fish D, Barbour AG. An ecological approach to preventing human infection: vaccinating wild mouse reservoirs intervenes in the Lyme disease cycle. *Proc. Natl. Acad. Sci. USA*. 2004; 101:18159–18164. [PubMed: 15608069]
- Wilson ML, Spielman A. Seasonal activity of immature *Ixodes dammini* (Acari, Ixodidae). *J. Med. Entomol.* 1985; 4:408–414. [PubMed: 4045938]

Fig. 1.

Nest box deployed in the study site. Boxes were cable tied to trees with the entrance facing the tree. Left panel, cheap pine bluebird box (“small” box). Right panel, larger hardwood construct (“large” box).

Fig. 2.

Bait preference in the presence of rodent chow ad libitum. Mice were housed in either small (standard shoebox) or large (rat size) cages. A, peanut butter scent, rough surface; B, peanut butter scent, smooth surface; C, blueberry scented, rough surface. Starting weights for baits differed between formulations. Absence of bar indicates that no bait remained. Error bar represents one-sided 95% confidence interval.

Fig. 3. Fluorescent staining of vibrissae; epifluorescence microscopy, $\times 100$. Left panel, non-stained (no bait ingested) vibrissa. Right panel, representative fluorescent band on vibrissa indicating that bait had been ingested. Bar = 500 microns.