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Original Article

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.

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 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

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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., 1991; 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, 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, $5 \times 5 \times 0.75$ 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. leucopous fusus*, 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 and Spielman, 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. On Grid 1, a commercially available (Mill Stores, Westboro, MA), inexpensive bluebird box ($20 \times 15 \times 15$ cm) made of unfinished pine was used. On Grid 2, a custom made, larger nest box ($25 \times 20 \times 20$ cm) 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 $P < 0.05$.