VECTOR-BORNE AND ZOONOTIC DISEASES
Volume 9, Number 4, 2009
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DOI: 10.1089/vbz.2008.0182

ORIGINAL ARTICLE

The United States Department of Agriculture Northeast Area-Wide Tick Control Project: History and Protocol

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Abstract

The Northeast Area-wide Tick Control Project (NEATCP) was funded by the United States Department of Agriculture (USDA) as a large-scale cooperative demonstration project of the USDA—Agricultural Research Service (ARS)–patented 4-Poster tick control technology (Pound et al. 1994) involving the USDA—ARS and a consortium of universities, state agencies, and a consulting firm at research locations in the five states of Connecticut (CT), Maryland (MD), New Jersey (NJ), New York (NY), and Rhode Island (RI). The stated objective of the project was "A community-based field trial of ARS-patented tick control technology designed to reduce the risk of Lyme disease in northeastern states." Here we relate the rationale and history of the technology, a chronological listing of events leading to implementation of the project, the original protocol for selecting treatment, and control sites, and protocols for deployment of treatments, sampling, assays, data analyses, and estimates of efficacy.

Key Words: 4-Poster—*Amblyomma americanum*—blacklegged tick—*Ixodes scapularis*—lone star tick—Lyme disease—white-tailed deer.

Development of Host-Targeted Tick Control Technologies for White-Tailed Deer and Other Wild Ungulates

Although they are only marginal hosts for the cattle tick, *Rhipicephalus* (*Boophilus) annulatus* (Say), and the southern cattle tick, *Rhipicephalus* (*Boophilus) microplus* (Canestrini), growing populations of white-tailed deer, *Odocoileus virginianus* (Zimmermann), and other wild ungulates along the Texas–Mexico border seriously threaten efforts of the USDA–Animal Plant Health Inspection Service(APHIS)–Veterinary Services (VS)–Cattle Fever Tick Eradication Program (CFTEP) to maintain eradication of these ticks from the continental United States. The presence of native and exotic ungulates poses a threat of uncontrolled reintroduction, dissemination, and maintenance of cattle fever ticks on white-tailed deer and other wild ungulates and the potentially fatal disease agents *Babesia bigemina* (Smith and Kilbourne) and *B. bovis* Starcovici that the ticks could transmit to U.S. cattle. Consequently, in 1989 the USDA—ARS mandated that scientists at the Knipling-Bushland U.S. Livestock Insects Research Laboratory (KBUSLIRL) in Kerrville, TX, should initiate an intensive research program to develop technology to control ticks feeding on wild ungulates. Because white-tailed deer are the primary large wild hosts for parasitic stages of lone star ticks, *Amblyomma americanum* (L.), this tick–host association (Patrick and Hair 1978, Bloemer et al. 1986, 1988) was selected as a model for developing host-targeted tick control strategies for wild ungulates. Since it is neither logistically nor economically feasible to capture and manually treat these wild animals, research approaches must focus on the development of systemic and topical chemical self-treatment technologies.

Systemically active ivermectin-medicated whole kernel corn was formulated, tested, and first shown to be highly effective in controlling lone star ticks feeding on white-tailed deer housed in stalls under controlled laboratory conditions (Miller et al. 1989). Later, this technology also proved highly efficacious against both parasitic and nonparasitic (free-living) cohorts of ticks in field trials conducted in large (ca. 19 ha) deer-fenced pastures (Pound et al. 1996). The technology was also used successfully to eradicate two long-term infestations of *R.* (*B.) annulatus* that were maintained by wild elk (*Cervus elaphus* L.) on the 2630 ha Apache Ranch (Webb County, TX) and by white-tailed deer on 8900 ha of the 16,190 ha Catarina Ranch (Webb County, TX). These adjacent,

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individually game-fenced ranches lie within the cattle fever tick quarantine zone north of Laredo in south Texas along the Texas–Mexico border.

Because ivermectin present in venison of deer treated systemically with medicated corn is not approved either by the U.S. Food and Drug Administration (FDA) or by the U.S. Environmental Protection Agency (EPA) for consumption by humans in the United States, research was initiated to develop topical self-treatment technologies utilizing chemical acaricides that would remain on the surface of the skin, would not contaminate the meat, and therefore would require no withdrawal period from application to consumption of the venison. This would permit tick control efforts on white-tailed deer to be implemented during hunting seasons. The first such topical self-treatment technology developed at the KBUSLIRL was a self-treatment bait station that consisted of a central bin to hold untreated clean whole kernel corn as bait and two feeding/treatment stations at either end. The proprietary design of the stations forced deer to rub the sides of the head, neck, and ears against two short vertical acaricide impregnated applicator rollers as they accessed the bait. Having two vertical rollers on either end, the device was dubbed the 4-Poster and was patented by ARS in 1994 (Pound et al. 1994, 2000a). Pigmented powder temporarily used on the applicator rollers simulated the locations that acaricide would be applied to the heads, necks, and ears of white-tailed deer inhabiting the KBUSLIRL. Subsequently, a field trial designed to evaluate efficacy using an oily formulation of the acaricide, amitraz, applied to white-tailed deer to control parasitic and free-living field populations of lone star ticks in large game-fenced pastures demonstrated that the technology was highly efficacious. Efficacy after the third year of treatment was 91.9% and 93.7%, against nymphs and adults, respectively (Pound et al. 2000b), which compared favorably with results from a similar 3-year trial in which the systemic ivermectin-medicated bait technology provided 92.0% and 92.6% control, also against nymphs and adults, respectively (Pound et al. 1996).

Concept and Organization of the USDA NEATCP

The obvious prior successes in controlling lone star ticks suggested that the medicated bait and 4-Poster technologies might also be applied in the control of other ticks for which white-tailed deer were very important or essential hosts. This similar and perhaps even stronger parasite/host relationship exists with populations of blacklegged ticks (*Ixodes scapularis* Say) for which white-tailed deer are the keystone host for the adult ticks (Main et al. 1981, Spielman et al. 1985, Barbour and Fish 1993).

In September 1996, a meeting was held at the USDA–ARS Beltsville Agricultural Research Center (BARC) (Beltsville, MD) to discuss the feasibility of using the two host-targeted tick control technologies in a large area-wide field project to attempt control of free-living populations of blacklegged ticks. Although most of those present were ARS scientists and administrators, representatives from university and military research groups were also in attendance. It was decided to limit intervention only to use of the 4-Posters and the 2% oily formulation of amitraz marketed as Point-Guard® (Hoechst Roussel Vet, Warren, NJ). Although an oily formulation of permethrin was also available and both permethrin and amitraz are highly efficacious against ticks, amitraz was selected because it is somewhat less toxic to insects. This factor was considered important in gaining acceptance of the technology by homeowners, their associations, regulatory agencies, and others. It was also decided to attempt control of the ticks in several different ecological environments throughout the most seriously tick-infested regions of the northeastern United States from MD through RI and perhaps into MA. In addition, control efforts initially were to be focused within populated community areas and not in unpopulated forests, parks, wilderness areas, etc. Names of scientists in each state with experience and expertise in blacklegged tick biology and field sampling were listed, and plans were made to visit several scientists and potential research sites.

During mid-November 1996, Community Tick Control Strategy Meetings were held at the Calder Ecology Center of Fordham University in Armonk, NY, the Department of Epidemiology and Public Health of the Yale School of Medicine in New Haven, CT, Otis Air National Guard Base, MA, and the University of Rhode Island in Kingston, RI. Originally, research sites in NJ, NY, CT, and RI were selected, and a meeting with principal investigators from these states and BARC convened during May 1997 at the Calder Ecology Center. Also in attendance were representatives of Hoechst Roussel Vet. By July, principal investigators submitted individual proposals that identified potential research sites, sampling techniques, treatment schedules, methods of efficacy evaluation, and other factors. The knowledge, ideas, and experience sequestered from meeting discussions, individual proposal submissions, and prior field trial studies of the 4-Poster technology led to completion of a comprehensive protocol for the USDA NEATCP during August 1997.

The Specific Objective

The stated purpose of this project was to determine the efficacy of Point-Guard (2% amitraz) applied to white-tailed deer by the USDA-ARS patented 4-Poster treatment device in reducing the risk of Lyme disease in endemic communities of the northeastern United States. A high percentage of adult *I. scapularis* feed on white-tailed deer. Controlling adult *I. scapularis* on deer would reduce numbers of free-living ticks that transmit the causative agent of Lyme disease, *Borrelia burgdorferi* Johnson, Schmid, Hyde, Steigerwalt, and Brenner, and other tick-borne pathogens to humans (Childs and Fish 2009). Populations of *A. americanum* would be affected similarly at sites where they occurred, which would further reduce the risk of pestilence and tick-borne disease transmission, for example, *Ehrlichia chaffeensis* and *Ehrlichia ewingii* (Childs and Paddock 2003).

Project Locations

The five sites that were selected differed in factors such as physical environment, structure of plant communities, diversity, and density of vertebrate species, and landscape of the properties on which homes were located (Fig. 1). At two of the sites, MD and NJ, most of the area was natural forest with few homes, whereas the remaining sites were located in populated community areas, as specified in the organizational plan. The sites were as follows: (1) near the town of Old Lyme, New London County, CT; (2) within the secured confines of Naval Weapons Station Earle, an active military base

FIG. 1. Map showing approximate locations (*) of research sites for the USDA NEATCP in MD, NJ, NY, CT, and RI. USDA NEATCP, United States Department of Agriculture Northeast Area-Wide Tick Control Project; MD, Maryland; NJ, New Jersey; NY, New York; CT, Connecticut; RI, Rhode Island.

in Monmouth County, NJ; (3) near the town of Bedford, Westchester County, NY; (4) in and around the town of Narragansett, Washington County, RI, and (5) a residential site including a golf course and gun club near Loch Raven, Baltimore County, MD. In addition to the Loch Raven location, funding was approved by ARS to support two additional project locations at BARC in Beltsville, Prince George's County, MD, and Gibson Island, Anne Arundel County, MD.

Site Selection Criteria

The primary objective was to assess effectiveness of the 4-Poster technology in reducing populations of free-living nymphs within a Core Treatment Area that was centrally located within a Treatment Site and to evaluate results by comparing tick populations in the Core Treatment Area to those in a similar but separate Control Site (Fig. 2). Core Treatment Areas would be approximately 518 ha (= 1280 ac or 2 mi^2) each, and efforts would be concentrated on controlling ticks within these areas. The 4-Poster devices would be deployed only within a Core Treatment Area. The selected Treatment and Control Sites were more circular than elongate, and Control Sites were to be similar in size to Core Treatment Areas. Because acaricide was being applied to free-ranging deer, the precise region in which tick control occurred would depend on movement and daily activity of deer that used the devices. Therefore, a Treatment Site would be selected that was large enough to contain both the 518 ha Core Treatment Area and enough peripheral area so that a gradient of control outward from the Core Treatment Area could be demonstrated.

Sizes and shapes of Treatment Sites were to vary somewhat among research locations to accommodate individual geographic characteristics of the regions surrounding the Core Treatment Areas and to demonstrate decreasing gradients of

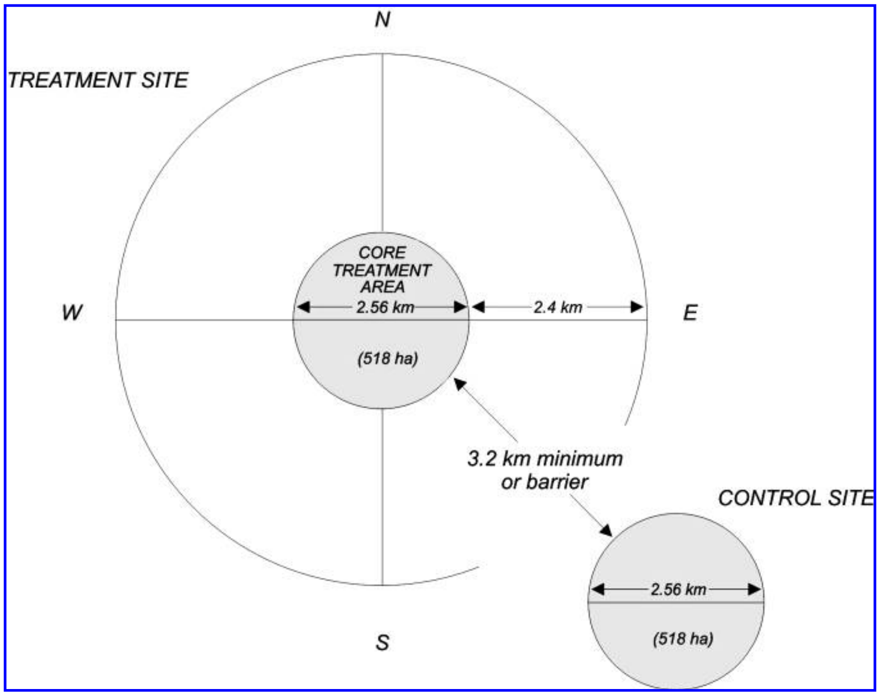

FIG. 2. Diagram illustrating the basic parameters of Treatment and Control sites used in the USDA NEATCP.

efficacy at the outer margins of Core Treatment Areas. Control Sites would be selected that either would be a minimum of 3.2 km (=2 miles) from the outer margins of the Core Treatment Sites or they would be separated from the Core Treatment Sites by a physical barrier, such as an impenetrable deer-fence or a freeway, to assure no cross-over effects from treated deer entering the untreated Control Sites. The basic comparison at each research location was to evaluate efficacy of the full intervention effort (devices, bait, and acaricide) versus no intervention at all (no devices, bait, or acaricide). Control Sites were to be selected that presumably would not be treated for ticks by homeowners, professional pest control operators, or others during the period of the study. To ensure that Control Sites would remain representative of natural populations of ticks, no 4-Poster devices or feed would be deployed at these sites.

Treatment Schedule

To allow deer to acclimate to the devices and begin using them on a regular basis, the 4-Poster devices with corn, but without acaricide, were to be placed in the field on or around 15 August in CT, NY, and RI, and 1 September in MD and NJ (but see individual site reports for deviations in protocol). During the first 30 days thereafter, placement of the devices was to be adjusted to optimize usage by deer. Optimization would be based on several factors, including the rate of consumption of corn, direct observation of deer and other animals near the devices, the amount of hair and degree of soiling of the rollers (an indication of usage), and spatial analysis of corn consumption data for individual devices.

Although treatment dates were subject to change as a result of unusual or extreme weather patterns, applications of Point-Guard would occur yearly from 15 September to 15 December and then begin again from 15 March to 15 May in NY, CT, and RI for a total of 151 treatment days. Applications in MD and NJ would be from 1 October through 15 December, resume on 15 March and continue though 31 August for a total of 244 treatment days, and would include late spring and summer months when lone star ticks generally feed on white-tailed deer. Treatments at any individual location, however, could be temporarily suspended during periods of tick inactivity that occurred when air temperature remained below 7.2°C (45°F) for extended periods or when snow cover exceeded 2.5 cm. Life cycles of ticks in the Northeast are quite long, with the blacklegged ticks requiring 2 or perhaps 3 years to develop from eggs to adults, and lone star ticks requiring perhaps 2 years. Initial deployment of the 4-Poster devices and treatment of deer in MD was delayed ca. 8 months to select study sites and to permit collection of pretreatment tick and deer baseline population data.

A minimum of 4 years of treatment was originally planned to demonstrate efficacy on free-living tick populations. Since this technology had never before been tested under unfenced or otherwise unrestricted field conditions, it was considered possible that unforeseen problems could compromise the first year of treatment. Activities conducted during the fifth year were to include final assessment of efficacy and technology transfer. Subsequently, because the first year of treatment was considered to be less than optimal, it was agreed that treatments would continue during the fifth year, and afterward all sites would continue to sample densities of nymphal ticks for an additional 2 years both to determine efficacy of the fifth year of treatment and/or to discern population rebound rates.

Distribution and Management of 4-Poster Devices

The 4-Poster devices were to be deployed at a rate of 25 devices per Core Treatment Area (=ca. 1 device for each 20.7 ha or 51.2 ac), and they were to be strategically placed in attempts to treat all deer that inhabited or entered and left the Core Treatment Area (but see individual site reports for deviations in protocol). Placement of the devices was to be at the discretion of each principal investigator and was dependent upon the observed activities and movements of deer. Therefore, it was not necessary or even advisable to place them in a precise grid-like or transect pattern. Researchers would contact property owners in desired locations and obtain permission to install the devices. The intention was that devices were to be installed on private rather than public property to minimize the potential for vandalism and maximize use by deer.

During installation, devices were to be individually numbered and leveled end to end and side to side to assure proper flow of bait from the feeding ports. Precise Global Positioning System (GPS) coordinates would be obtained for each device, and they would be filled with twice-cleaned or recleaned whole kernel corn of the type commonly used in automatic deer feeding devices. Initially, corn would be spread liberally both on the adjustable overhanging plates of the 4-Posters and on the ground around the ends of the devices to entice the deer into finding the corn being dispensed from the feeding ports of the devices. After deer began using the devices regularly, this enticement procedure would be eliminated.

Information from individual state wildlife agencies estimated pretrial populations of roughly 22 deer per 259 ha (=1 mi^2) or 44 deer per Core Treatment Area in each location; therefore, it was anticipated that a total of approximately 220 deer would be treated during the first year of the study. Estimates of deer densities at individual locations would be updated during subsequent years at some sites as a result of aerial and other surveying methods that were to be made during the first year. As mentioned, preliminary studies showed that >97% efficacy against adult ticks feeding on white-tailed deer was achieved using approximately 1.6 mL of the 2% AI Point-Guard formulation of amitraz per deer per day.

Because distribution of deer would not be homogeneous throughout the Core Treatment Areas, and an individual deer could visit more than one device daily, acaricide actually would be applied to rollers in amounts considerably greater than the minimal estimate of 1.6 mL per deer per day. Initially, each roller would be treated with 25 mL of Point-Guard. Afterward, researchers were instructed to evaluate the relative amounts of pesticide on rollers weekly or more frequently, if needed, by wiping individual rollers with a brown paper towel or similar absorbent material to approximate the amount of oily residue. Wearing protective gloves, researchers using the specially adapted applicator guns would apply acaricide in increments of 5 mL to each of the rollers. During the second year, assays from hair coats of anesthetized deer showed less than effective quantities of acaricide. Therefore, acaricide application was increased to a rate of 1.75 mL per 0.45 kg (=1 lb) of corn consumed from each device since the previous visit. Dates, amounts of corn consumed, and amounts of acaricide applied were to be recorded at each visit for each individual 4-Poster.

Data Collection

4-Poster devices

To maximize use of the devices by deer, it likely would be necessary to adjust their placement within the Core Treatment Areas, and these decisions would be based primarily on knowledge or perception of relative use of the devices (see individual site reports for deviations in protocol). It was emphasized that precise records were to be maintained on the amount (weight) of corn placed in each device and volume of pesticide applied to the rollers. Previous studies in Texas had shown that, in the presence of abundant forage, white-tailed deer would consume only about one percent of their body weight in whole kernel corn per day. Thus, a rough estimate of the use of the device by deer could be determined from the rate of corn consumption. Graduated marks within the corn bin of each device permitted operators to estimate consumption rates of corn for individual devices. These values and GPS coordinates of individual devices could be analyzed using spatial analysis software to graph device usage and assist researchers in maximizing placement.

White-tailed deer

Because the behavior and ecology of these free-ranging deer were unknown variables, quantification of deer densities were to be made at each research site. Wildlife experts specifically trained in deer population ecology would periodically estimate deer density, the proportion of deer treated, and the density of ticks feeding on deer.

At most sites, deer population estimates would be conducted from December to February when there was >10 cm of snow cover on the ground. A helicopter would be used to fly transects covering both Treatment and Control Sites. Each transect would be the entire length of a Site and 150 m in width. Two observers would count all deer on each transect and record the number and distribution on aerial photographs. Variation among methods for conducting deer censuses is detailed in the individual site reports.

Proportions of deer using the devices would be determined by temporarily replacing acaricide treated rollers with rollers containing a reflective marking agent, such as glass microspheres in a petroleum jelly base that is reflective at night. This reflective marking agent allowed nocturnal observation using a spotlight that was easier to accomplish and perhaps more accurate than daylight counts of deer marked with fluorescent or other colored pigments. This technique also avoided having conspicuously marked deer present in suburban communities.

Abundance and distribution of ticks on deer would be determined by capturing and examining 12 deer (6 in the Core Treatment Area and 6 in the Control Area) during October and November. Wildlife biologists with expertise in handling, restraining, and examining wildlife would capture deer by remotely immobilizing them with a dart gun from a tree stand near a bait source. Immobilizing agents would include tiletamine HCl + zolazepam HCl (4.4 mg/kg), and xylazine HCl (2.2 mg/kg); yohimbine HCl (0.12 mg/kg) subsequently would be used to reverse the anesthesia. Anesthetized deer would be examined for ticks, and data including counts by stage, sex, state of engorgement, and location on the host would be recorded. Because of anesthetizing drug residues within the blood of recently darted deer, the deer would be ear-tagged informing persons who might later come in contact with the animals (i.e., from hunted or road-killed deer) to notify the appropriate agency and determine if sufficient time had elapsed between anesthesia and slaughter to allow safe consumption the venison. Ear-tagging also was to be used to prevent the resampling of previously captured deer. Deer could not be sampled at some sites as detailed in the individual site reports.

Tick sampling

Because of differences in vegetation types, densities, and other factors, specific methods of tick sampling varied among locations. All participating researchers, however, had expertise in sampling tick populations in their specific locations, and discussions among members of the consortium established sampling protocols that would assure collection of equivalent data sets. Adult, nymphal, and larval population estimates were to be assayed using either standard cloth drags, flags, or walking samples after which ticks were counted directly on light-colored clothing.

To assay efficacy of treatment efforts in Core Treatment Areas, 15 to 25 randomly dispersed plots would be selected having vegetative characteristics that were similar and typical of the type of habitat most likely to harbor ticks within both Core Treatment Areas and Control Sites. Each plot would be sampled either by dragging or flagging a total of 100 m^2 or by walking through shrubs and brush for 10 periods of 30 s and counting ticks clinging to white clothes after each period. The 15 to 25 plots would be sampled during as nearly the same period of time as possible, and the entire data set obtained would constitute a single sample. During anticipated periods of peak activity each year, adults and larvae would be sampled at least once. Because of their paramount importance in Lyme disease risk, nymphs would be sampled weekly for four weeks to include the week of anticipated peak activity.

To assay the extent to which treatment effects extended beyond the periphery of the Core Treatment Areas, Treatment Sites would be sampled at 0, 0.4, 0.8, and 1.2 km intervals outward from the boundary of the Core Treatment Areas. These samples would be taken along transects in the approximate cardinal compass directions of north, south, east, and west outward from the geographic center of the Core Treatment Area for a total of 16 samples.

To determine rates of *B. burgdorferi* infection in free-living nymphs, sub-samples of 100 nymphs from each treatment site were to be sent to the Vector Ecology Laboratory at the Department of Epidemiology and Public Health of the Yale University School of Medicine to be assayed as described below.

Estimating Efficacy of Treatments

Assessment of tick sampling

For each location, calculations of efficacy would be based on annual comparisons of the densities of each life stage sampled in Core Treatment Areas versus the corresponding

Control Sites. Abbott's formula (Abbott 1925) would be used to calculate a simple percent corrected control of larvae, nymphs, and adult ticks, while other statistical analyses would be employed to ascribe appropriate levels of significance to these comparisons (variation among tick sampling and data analysis methods is detailed in the individual site reports). As explained, multiple sampling of tick density during peak periods would be obtained for nymphs, and the principal estimate of the efficacy of the treatment would be based on the relative reduction in the size of this cohort of the free-living population.

Assay of ticks for human pathogens

Ticks were to be assayed for human pathogens at the Yale School of Medicine. Assays conducted at this centralized single facility would provide consistent methodologies for comparison of pathogen prevalence in host-seeking ticks among each of the project locations. Also, a laboratory colony of *I. scapularis* would have the capability of providing both positive and negative controls for quality control of pathogen assays. Nymphal and adult *I. scapularis* would be assayed for *B. burgdorferi* and the agent of human granulocytic anaplasmosis, *Anaplasma phagocytophilum*. Although nymphal *I. scapularis* are the primary vectors of *B. burgdorferi* to humans, there is epidemiological evidence that both nymphal and adult *I. scapularis* are important vectors of *A. phagocytophilum* to humans. Because the focus of this study was *I. scapularis* and Lyme disease, *A. americanum* would not be assayed for the agent of human monocytic ehrlichiosis, *E. chaffeensis*.

Although adherence to these standardized protocols was high at each site, there was some inter-site variability among methods and data analyses; thus, the reader is encouraged to read the individual site reports that follow. Two additional articles by Brei et al. and Pound et al. provide a meta-analysis and a summary of results with conclusions, respectively, which provide a synthesis of the important findings of this joint effort.

Disclosure Statement

The 4-Poster device was developed and patented as a result of research by scientists of the USDA, ARS. Scientists J.M. Pound and J.A. Miller are two of the three inventors listed on the patent and receive nominal royalties through the USDA as per government regulations. J.E. George and D. Fish have no competing financial interests.

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