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Longitudinal Evaluation of an Educational Intervention for Preventing Tick Bites in an Area with Endemic Lyme Disease in Baltimore County, Maryland

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The authors attempted to determine whether a targeted educational intervention in an area with endemic Lyme disease could increase knowledge, positive attitudes, and reported behaviors related to tick bite prevention and consequently decrease tick bites, as measured by a biomarker of tick bites. Between April and September of 1999, 317 subjects in Baltimore County, Maryland, were randomized to receive either tick-related or general health-related educational materials bimonthly through the mail. At each of three clinic visits, participants completed a self-administered questionnaire and provided a serum sample. Anti-recombinant tick calreticulin antibody (ARTCA), measured in ng/µl, was used as a biomarker of tick bites. Linear and logistic regression analyses were used to determine 1) whether the educational intervention was associated with a change in knowledge, attitudes, and behaviors (KAB) and 2) whether change in KAB predicted change in ARTCA levels. Proportions of desired responses increased significantly among intervention subjects versus the comparison group on KAB measures related to examining the body for ticks and insect repellent use. Levels of ARTCA were low among all study subjects. Only six of 37 models exhibited a significant relation between change in a KAB variable and change in ARTCA levels over time. The behavioral intervention was associated with an increase in the KAB measures in the intervention group, but this change was not associated with change in ARTCA levels.

biological markers; intervention studies; Lyme disease; randomized controlled trials; tick-borne diseases

Abbreviations: ARTCA, anti-recombinant tick calreticulin antibody; DEET, *N*,*N*-diethyl-*m*-toluamide; KAB, knowledge, attitudes, and behaviors.

Lyme disease, which is transmitted through the bite of ticks from the *Ixodes ricinus* complex, is the most prevalent vector-borne disease in the United States (1). The increase in the incidence of Lyme disease domestically and worldwide corresponds to a trend of increasing incidence of tick-borne diseases generally in the United States (2–8). Suburbanization, growing deer populations, changing patterns of recreational activity, and warmer weather have been hypothesized to be factors in the rise of vector populations and vector-human interactions (2, 9, 10).

Currently, the Centers for Disease Control and Prevention and most state health departments promote self-inspection for ticks, use of insect repellents, and wearing of light-colored clothing and long pants in fields and wooded areas to prevent tick bites (11, 12). A vaccine against Lyme disease was marketed in the United States for 3 years (12, 13) but

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was withdrawn by the manufacturer in February 2002 because of low demand (14). The vaccine was 76 percent effective in preventing symptomatic "definite" Lyme disease after three doses. It did not provide protection against other tick-transmitted pathogens, so other methods of personal protection continued to be recommended for populations at risk of tick-borne disease.

Several cross-sectional studies have investigated current levels of knowledge and behavior in areas with endemic Lyme disease and have found that only 30–45 percent of persons admit currently practicing some form of personal protection against tick bites (15–18). The effectiveness of these behaviors in preventing tick bites has not been evaluated. Human antibody response to tick salivary gland proteins has been shown to be a biologic marker of tick exposure in both experimental studies in animals and observational studies in humans (19–25), and it could be used to assess the effectiveness of an intervention designed to prevent tick bites.

Antibodies to whole salivary gland extract (anti-tick saliva antibodies) and antibodies to a recombinant tick saliva protein (recombinant tick calreticulin) have been evaluated (26–28). Calreticulin is produced in tick saliva after approximately 3 days of tick feeding, so anti-recombinant tick calreticulin antibody (ARTCA) is a biomarker for tick bites of longer duration (29). In a study of tick bite recipients in Westchester County, New York, a tick engorgement index was associated with ARTCA levels, and persons without a previous tick bite had significantly lower ARTCA levels than did tick bite recipients (28). Studies have also reported that risk and preventive behaviors are associated with ARTCA and anti-tick saliva antibody levels (25, 27, 29).

The goal of this population-based randomized intervention study was to determine whether a targeted behavioral intervention in an area with endemic Lyme disease could increase the level of knowledge, attitudes, and behaviors (KAB) related to tick bite prevention and consequently decrease tick bites. Changes in KAB measures were then evaluated as predictors of change in ARTCA levels over time, to determine whether the intervention had an impact on the incidence of tick bites during the study.

MATERIALS AND METHODS

Study design and study population

The study site was an area in Baltimore County, Maryland, north of Baltimore City. In 1997, Baltimore County reported an average Lyme disease rate of 13 cases per 100,000 population (K. Damewood, Maryland Department of Health and Mental Hygiene, personal communication, 1998), ranging from a low of 4.35 per 100,000 to a high of 71.52 per 100,000 in certain high-risk zip codes (30). A total of 317 subjects aged 18–65 years were recruited by random digit dialing and enrolled between February 5, 1999, and April 1, 1999. Subjects were informed that the study concerned Lyme disease in Baltimore County, and they were preferentially recruited from Baltimore County zip codes identified as highly endemic for the disease using a method based on geographic information systems (30). During the initial telephone call to a potential subject, residential zip code and possible risk of tick exposure were confirmed through questions on type of residential area and the presence of deer and ticks in the area.

Subjects were randomly assigned to either the intervention group or the comparison group and were frequency-matched according to age, gender, and zip code. Because of concern about anticipated lower follow-up rates in the comparison group, 169 subjects (53.5 percent) were randomly assigned to the comparison group and 148 (46.7 percent) were assigned to the intervention group. Each group received a mailing every 2 weeks from April 1999 to September 1999 (table 1), for a total of 10 mailings. The intervention group received literature and tools designed to educate and to enable each individual to examine his or her entire body for ticks, identify and remove ticks found during these body checks, and apply the repellent *N*,*N*-diethyl-*m*-toluamide (DEET) to the skin and the acaricide permethrin to the clothing. The comparison group received health-related educational materials and tools unrelated to tick bite prevention, such as toothbrushes and booklets containing recipes for low-fat foods (table 1).

Participation was voluntary. Each subject provided written informed consent and was compensated $25.00 for each clinic visit. The study was approved by institutional review boards at the Johns Hopkins School of Hygiene and Public Health, the Maryland Department of Health and Mental Hygiene, and the Centers for Disease Control and Prevention.

Data collection

During the three clinic visits, participants in both groups were asked to complete a self-administered 20-page questionnaire, which was then checked for completeness and accuracy, and to provide a serum sample, which was obtained by a phlebotomist. The first clinic visit was completed between February 5 and April 1, 1999; the second was completed between June 10 and July 30, 1999; and the last was completed between September 16 and October 30, 1999. The questionnaire contained questions related to residential, recreational, and occupational tick bite risk; history and knowledge of tick bites and Lyme disease; and knowledge and use of tick bite prevention methods. Other questions pertaining to Lyme disease included information on whether a subject knew someone with the disease or had been vaccinated against, tested for, or treated for Lyme disease. Throughout the intervention period, subjects were asked to return 10 brief checklists, included in each mailing, within 1 week of receipt. The checklists contained questions related to tick exposure and use of the intervention materials included in the previous mailing.

Educational intervention

From February to December of 1998, study investigators conducted formative research through key informant interviews with local health and natural resource officials. They also conducted semistructured interviews and created focus groups with persons recruited from the study area. Examination of the entire body for ticks after outdoor activities, removal of ticks identified during these body checks, and use of tick repellents and acaricides were identified as the most

TABLE 1. Intervention and comparison group materials ("Protect–Detect–Remove") mailed to subjects in a study of tick bites and Lyme disease (*n* = 317), Tick Bite Prevention Project, Baltimore County, Maryland, 1999

Date of mailing	Intervention group materials	Comparison group materials
April 9	Lyme disease brochure (Centers for Disease Control and Prevention)	*Diet and Health Recommendations for Cancer Prevention* booklet (National Cancer Institute)
	Brochure explaining how to examine the body for ticks after outdoor activity	
	Handheld mirror for conducting tick checks	
	Magnet for hanging up brochure	
April 19	Tick removal brochure	*Getting What You Want from Exercise* and *Fitness Self-Test* brochures
	Tweezers for tick removal	
	Magnet for hanging up brochure	
May 3	Permethrin acaricide sample*	*Stress* and *5 Smart Steps to Less Stress* brochures
	Permethrin acaricide brochure	
May 17	*Questions You Asked Us about Ticks and Their Habits* brochure	*Taking Care of Your Teeth and Gums* brochure (American Dental Association)
	Questions You Asked Us about Repellents and Pesticides brochure	Toothbrush†
		Dental floss‡
May 31	DEET§ repellent sample¶	*Choosing a Toothpaste* brochure
	DEET repellent brochure	Toothpaste sample#
June 14	Permethrin** or DEET sample††	*Water on Tap: A Consumer's Guide to the Nation's Drinking Water* (Environmental Protection Agency)
		Bottled water‡‡
July 1	Lyme Disease Vaccine Fact Sheet	Outdoor magazine§§
July 20	Tweezers for key chain	*Incredible Food Facts* and *Eating Well and Looking Good* brochures
	Tick identification card (US Army)	
August 9	Intervention summary sheet	*The Sun, UV, and You: A Guide to SunWise Behavior* booklet
August 31	T-shirt with logo and trademark	T-shirt with logo and trademark
	Two stickers with trademark	Two stickers with trademark
		Intervention group materials

* Permethrin Tick Repellent spray with 0.50% permethrin; donated by Sawyer Products, Tampa, Florida.
† Butler G-U-M toothbrush; donated by the John O. Butler Company, Chicago, Illinois.
‡ Butler G-U-M dental floss; donated by the John O. Butler Company, Chicago, Illinois.
§ DEET, *N*,*N*-diethyl-*m*-toluamide.
¶ Ben's Backyard 2-ounce lotion with 23.75% DEET; donated by the Tender Corporation, Littleton, New Hampshire.
\# Colgate Total toothpaste; donated by the Colgate-Palmolive Company, New York, New York.
** Permanone Repel Tick and Mosquito Repellent for Clothing unscented spray; donated by the Wisconsin Pharmacal Company, Inc., Jackson, Wisconsin.
†† Ben's Backyard 6-ounce Eco-spray with 23.75% DEET; donated by the Tender Corporation, Littleton, New Hampshire.
‡‡ Deer Park bottled water; donated by The Perrier Group of America, Greenwich, Connecticut.
§§ *Outside*; donated by *Outside* Magazine, Santa Fe, New Mexico.

feasible and acceptable sets of behaviors for residents of the study area. Consequently, a tailored, comprehensive educational intervention designed to promote these three sets of behaviors was developed and pretested. The resulting intervention, entitled "Protect–Detect–Remove," consisted of print materials and products relevant to tick bite protection distributed through the mail over a 10-week period. The products mailed to participants included DEET, permethrin, laminated hanging 3″ × 8″ (8 cm × 20 cm) cards with pictorial and textual instructions on how to perform self-inspection of the body for ticks (hereafter termed a "tick check") and how to remove a tick, a handheld mirror, and various types of tweezers for removing attached ticks (table 1). Exposure to various components of the intervention was assessed with the questionnaire completed at each study visit.

Laboratory methods

Recombinant tick calreticulin was supplied by Dr. Bruce Ritchie of the University of Alberta (Edmonton, Alberta, Canada). ARTCA levels were measured by enzyme-linked immunosorbent assay using previously published methods (27, 28). Briefly, serologic assays were conducted without knowledge of study subject allocation, and all samples for a given study subject were assayed on the same plate in duplicate. Enzyme-linked immunosorbent assay plates were coated with 0.2 μg of recombinant tick calreticulin (in 100 μl of phosphate-buffered saline) per well overnight at 4°C. The plates were blocked with blotto (2 percent nonfat milk in phosphate-buffered saline) for 90 minutes at 37°C. Sera were diluted 1:200 in blotto, and quantities of 100 μl per well were incubated overnight at 4°C. After washing, a 1:1,000 dilution of goat anti-human immunoglobulin G conjugated to horseradish peroxidase in blotto was added to the plates and incubated for 75 minutes at 37°C. The plates were developed with 2,2-azino-di(3-ethylbenzthiazoline) sulfonic acid substrate, and the absorbance was measured at 405 nm. The estimated amount of specific human ARTCA (ng/μl) was determined on the basis of a kinetic measure of ARTCA enzyme-linked immunosorbent assay absorbance. The absorbance was adjusted for background (the absorbance from wells without recombinant tick calreticulin) and was then converted to nanograms of human immunoglobulin G per microliter of human serum using a standard curve of known human immunoglobulin G quantities. Sample assays were repeated until the duplicates were within ±15 percent, and assays for all subjects who evidenced changes in ARTCA levels across visits were repeated. Consequently, 404 samples (47.3 percent) were assayed once, 335 samples (41.6 percent) were assayed twice, and 95 samples (11.1 percent) were assayed three or more times.

Statistical analysis

The goals of the data analyses were to investigate predictors of change in KAB variables between groups over time and consequently to assess which KAB variables predicted change in ARTCA levels over time in the population as a whole. Differences in gender, educational level, type of residence, area of residence, and time spent outdoors at various locations between the two groups were assessed using either a chi-squared test and Kendall's tau *b* or a *t* test. Univariate and bivariate analyses and factor analysis were performed using SPSS, version 7.5 (31). Linear and logistic regression modeling using generalized estimating equations was performed using SAS for Windows, version 6.12 (32).

Modeling of KAB variables. Principal components analysis with varimax rotation was employed to reduce the number of KAB variables and to build reliable unidimensional scales for constructs such as self-efficacy in preventing tick bites and perceived safety of repellents (33, 34). There were no existing scales in the literature for these constructs. The reliability of the self-efficacy scale was evaluated, and the scale had an alpha value equal to 0.71. This scale and other scales, derived from Likert responses, were treated as continuous variables in subsequent analyses.

Linear regression was used to identify predictors of change for each continuous or interval measure over time, while logistic regression was used to identify predictors of change for each dichotomous measure over time. Since each regression model involved repeated measures of outcome variables in individuals over time, generalized estimating equations were utilized to account for the intrasubject correlation among repeated measures using an exchangeable correlation assumption (35). Regression coefficients used in equations with continuous outcomes may be interpreted as predictor effects on the outcome measures over time. Standard errors correctly accounted for correlations among repeated measures within subjects. Regression coefficients used in equations with dichotomous outcomes estimate the relative odds of exhibiting the outcome between groups at the different study visits. Two questions (resulting in the creation of three variables that concerned self-reporting of finding ticks on the body and tick bites) on the visit 1 questionnaire assessed lifetime history rather than a period of a few months as on the visit 2 and visit 3 questionnaires. The change from visit 1 to subsequent visits must be interpreted with caution for these outcome variables.

The base model for all generalized estimating equations for KAB measures consisted of indicator variables for visits 2 and 3, age, sex, race, study group (intervention vs. comparison), and cross-products related to visit and study group. This model allowed us to separately assess changes in levels of the KAB variables by group from visit 1 to visit 2 and from visit 1 to visit 3.

Modeling of ARTCA levels. The distribution of ARTCA levels was examined before modeling. ARTCA levels were natural log-transformed, and the distribution of the residuals was examined for normality. The base models for the ARTCA analysis included a single KAB variable, indicator variables for visits 2 and 3, age, sex, race, and cross-products related to KAB variable and visit. This model allowed us to separately assess changes in ARTCA levels associated with changes in KAB predictor variables for visit 1 to visit 2 and visit 1 to visit 3.

RESULTS

Characterization of study subjects

The mean age of the comparison subjects was 44.3 years (standard deviation, 11.7; range, 18–65 years), and the mean age of the intervention subjects was 43.5 years (standard deviation, 10.6; range, 18–64 years) ($p > 0.05$) (table 2). The mean duration of having lived at the current residence for comparison subjects was 9.5 years (standard deviation, 9.1), with a range of 1 month to 40 years. The corresponding value for the intervention group subjects was 9.5 years (standard deviation, 8.4), with a range of 1 month to 35 years ($p > 0.05$). There were no significant differences between the two groups in terms of gender, educational level, type of residence, area of residence, or amount of time spent outdoors at various locations. A total of 288 subjects (90.9 percent) returned for the second visit, and 258 (81.4 percent) returned for the third visit. A total of 239 subjects (75.4 percent) provided three blood specimens; 55 (17.4 percent) provided two specimens; 22 (6.9 percent) provided one specimen; and one (0.3 percent) provided no blood.

TABLE 2. Self-reported demographic characteristics of study participants at baseline, by study group, Tick Bite Prevention Project, Baltimore County, Maryland, 1999

Variable*	Intervention group		Comparison group	
	No.	%	No.	%
No. of subjects	148	46.7	169	53.3
Age (years) of females				
18–25	5	3.4	1	0.6
26–35	16	10.8	26	15.4
36–45	41	27.7	35	20.7
46–55	24	16.2	26	15.4
56–65	9	6.1	23	13.6
Missing data	0	0.0	0	0.0
Total	95	64.2	111	65.7
Age (years) of males				
18–25	3	2.0	5	3.0
26–35	12	8.1	9	5.3
36–45	9	6.1	21	12.4
46–55	20	13.5	11	6.5
56–65	8	5.4	12	7.1
Missing data	1	0.7	0	0.0
Total	53	35.8	58	34.3
Educational level				
Less than high school	10	6.8	8	4.7
High school graduate	49	33.1	66	39.1
College graduate	50	33.8	60	35.5
More than college	39	26.3	35	20.7
Type of residential area				
Rural	51	34.4	52	30.8
Suburban	96	64.9	114	67.4
Urban	1	0.7	3	1.8
Type of residence				
Apartment	19	12.8	22	13.0
Townhouse	16	10.8	28	16.6
Unattached house	113	76.4	119	70.4
Features surrounding residence				
Yard	143	96.6	162	95.9
Garden	118	79.7	127	75.1
Deer	109	73.6	128	75.7
Ticks	116	78.4	129	76.3
>1 acre of woods within 0.5 mile (0.8 km)	132	89.2	149	88.2
>1 acre of fields within 0.5 mile (0.8 km)	106	71.6	126	74.6
Mean amount of time spent outdoors (hours/week)				
Around house	14.3 (11.7)†		13.9 (12.3)	
At work	5.0 (10.3)		4.9 (12.1)	
Outdoor recreation	6.8 (6.5)		7.2 (9.6)	

* No differences were found to be significant between groups at $p = 0.05$ using the chi-squared test and Kendall's tau *b* or the *t* test.

† Numbers in parentheses, standard deviation.

TABLE 3. Distribution of selected self-reported measures related to ticks and Lyme disease among participants in the intervention and comparison groups, by study visit, Tick Bite Prevention Project, Baltimore County, Maryland, 1999

Variable	Description of variable	Intervention group						Comparison group					
		Visit 1		Visit 2		Visit 3		Visit 1		Visit 2		Visit 3	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
No. of subjects		148	100	134	90.5	126	85.1	169	100	156	92.3	129	76.3
General knowledge of ticks and Lyme disease													
XPROMNTH	Knowing the months in which one should begin protecting oneself from ticks	109	73.6	107	79.9	95	75.4	125	74.0	109	69.9	93	72.1
XDAYS	Knowing the amount of attachment time needed for transmission of bacteria	12	8.1	116	86.6	96	76.2	12	7.1	33	21.2	26	20.2
XSMALL	Knowing the size of the ticks which cause the most disease	102	69.9	97	72.4	78	61.9	112	66.3	115	73.7	96	74.4
*Knowledge of and reported behaviors related to tick checks**													
CHCKSELF	Reporting checking oneself for ticks	105	70.9	124	92.5	113	89.7	124	73.4	134	85.9	104	80.6
FNDTICK1	Reporting finding few ticks on oneself†	104	70.3	25	18.7	7	5.6	97	57.4	33	21.2	18	14.0
FNDTICK2	Reporting finding many ticks on oneself†	28	18.9	11	8.2	5	4.0	44	26.0	20	12.8	3	2.3
TICKBITE	Reporting having had a tick bite†	94	63.5	10	7.5	4	3.2	98	58.0	17	10.9	8	6.2

Table continues

The study subjects were enrolled from 42 different zip codes. Eleven zip codes (21030, 21093, 21111, 21117, 21120, 21131, 21136, 21152, 21208, 21234, and 21286) accounted for 130 (76.9 percent) of the comparison group subjects and 114 (77.0 percent) of the intervention group subjects. Randomization by zip code was successful in that there was no difference in the distribution of comparison and intervention group subjects by zip code ($p = 1.0$).

Crude analysis of change in KAB

KAB variables were categorized into three groups: general knowledge of ticks and Lyme disease; knowledge of and reported behaviors related to tick checks; and knowledge of and reported behaviors related to tick repellants and acaricides (table 3). The proportion of the intervention group with the desired response (e.g., more repellent use, fewer reported tick bites) increased for all KAB measures between visit 1 and visit 2 except for those comparing lifetime history with the period between visit 1 and visit 2 (table 3). Proportions of desired responses decreased slightly for many measures between visit 2 and visit 3, except for reported checking for ticks at work, familiarity with DEET and permethrin, and reported use of a permethrin-containing product. The proportion of the intervention group with the desired response was higher than the proportion of the comparison group for all KAB measures between visit 1 and visit 2 and between visit 2 and visit 3, except for variables related to knowledge of the size of the ticks causing the most disease and reported self-reports of finding ticks on oneself and tick bites.

The proportion of the comparison group knowing or reporting many attitudinal or behavioral measures also increased between visit 1 and visit 2, but to a lesser extent than in the intervention group. Furthermore, the proportion of the comparison group with the desired KAB responses continued to increase from visit 2 to visit 3, particularly on measures related to methods for tick checks and knowledge of repellents.

Modeling change in KAB variables over time

The most significant change in KAB measures between groups occurred between visit 1 and visit 2 on measures related to tick-check methodology and repellent knowledge and behavior. Selected models are presented in tables 4 and 5. Only for one measure of general knowledge concerning ticks and Lyme disease—knowledge of the amount of attachment time needed for transmission of disease-causing bacteria—was the odds ratio significantly higher for the intervention group than for the comparison group between all clinic visits.

TABLE 3. Continued

Variable	Description of variable	Intervention group						Comparison group					
		Visit 1		Visit 2		Visit 3		Visit 1		Visit 2		Visit 3	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
HCHKTCK	Reporting performing a tick check‡ at home	89	60.1	109	81.3	98	77.7	110	65.1	107	68.6	92	71.3
ACHKTCK	Reporting performing a tick check away from home	89	60.1	80	59.7	70	55.6	100	59.2	90	57.7	71	55.0
HANDHELD	Reporting use of a handheld mirror during tick check	19	12.8	88	65.7	78	61.9	22	13.0	27	17.3	23	17.8
Knowledge of and reported use of tick repellents and acaricides§													
DEETSKIN	Knowing that DEET¶ is used on the skin	50	33.8	109	81.3	101	80.2	64	37.9	86	55.1	72	55.8
XDEETPCT	Knowing the minimum % of DEET needed for DEET to be effective	4	2.7	78	58.2	58	46.0	7	4.1	4	2.6	6	4.7
SKINDEET	Reporting having used repellent containing DEET on the skin	18	12.2	53	39.6	47	37.3	26	15.4	20	12.8	21	16.3
XPRMCLTH	Knowing that permethrin is used on clothing	12	8.1	109	81.3	98	77.8	14	8.3	21	13.5	31	24.0
CLTHPERM	Reporting having used acaricide containing permethrin on clothing	5	3.4	37	27.6	41	32.5	3	1.8	3	1.9	5	3.9

* Selected measures from a total of 17.
† At visit 1, the questionnaire asked about lifetime history; at visits 2 and 3, the questionnaire asked about interim history (since the last visit).
‡ Self-inspection of the body for ticks.
§ Selected measures from a total of eight.
¶ DEET, *N*,*N*-diethyl-*m*-toluamide.

Intervention group subjects reported performing more tick checks during residential and recreational activities and performing more thorough tick checks than comparison group subjects at visits 2 and 3 as compared with visit 1. The odds of intervention group subjects' performing a tick check were 3.18 (95 percent confidence interval: 1.59, 6.37) between visit 1 and visit 2 and 1.94 (95 percent confidence interval: 0.99, 3.81) between visit 1 and visit 3 as compared with the comparison group (see table 4).

The most striking increases in odds ratios between visits for the intervention group concerned the repellent-related KAB measures. While the measure of reported change in wearing of a repellent was only significant between visit 1 and visit 2, all other repellent-related measures evidenced significant changes between all visits. The odds of intervention group subjects' being familiar with the repellent DEET were 42.59 (95 percent confidence interval: 15.09, 120.24) between visit 1 and visit 2 and 37.41 (95 percent confidence interval: 12.73, 109.89) between visit 1 and visit 3, as compared with the comparison group (data not shown in table 4). Similar odds ratios of 36.99 (95 percent confidence interval: 13.60, 100.63) between visits 1 and 2 and 36.50 (95 percent confidence interval: 12.15, 109.65) between visits 1 and 3 were associated with the measure of familiarity with permethrin.

Crude analysis of ARTCA levels

ARTCA levels evidenced only small changes over time. Overall, mean ARTCA levels increased 4.6 percent from visit 1 to visit 2 and 20 percent from visit 1 to visit 3. From visit 1 to visit 2, three persons evidenced at least a twofold decline, 13 persons showed at least a 50 percent decline, five persons showed at least a twofold increase, and 15 persons showed at least a 50 percent increase. From visit 1 to visit 3, six persons evidenced at least a twofold decline, 16 persons showed at least a 50 percent decline, 16 persons showed at least a 50 percent increase, and seven persons showed at least a twofold increase. Three comparison group subjects evidenced at least a threefold increase in ARTCA levels from visit 1 to visit 3, as compared with two intervention group subjects.

Modeling change in ARTCA levels over time

In linear regression analysis, only six of the 37 models exhibited a significant relation ($p < 0.05$) between change in a KAB variable and change in ARTCA levels. Selected models are presented in tables 4 and 5. The KAB variables that were associated with change in ARTCA levels were: knowing whether one had a tick bite (increase in ARTCA levels from visit 1 to visit 3), performing a tick check while away from home (decrease from visit 1 to visit 2), knowing that

TABLE 4. Associations of study group status (intervention group vs. comparison group) with changes in knowledge, attitudes, and reported behaviors regarding ticks and Lyme disease from study visit 1 to subsequent study visits, Tick Bite Prevention Project, Baltimore County, Maryland, 1999*

KAB† variable	Description of KAB variable	Model (change in KAB variable between visits)‡	Model with KAB variable used as the outcome			Model (change in ARTCA† variable associated with change in KAB variable)§	Model with ARTCA variable used as the outcome		
			Odds ratio¶	95% confidence interval	*p* value		β coefficient	Standard error	*p* value
			General knowledge of ticks and Lyme disease						
XPROMNTH	Knowing the months when one should begin protecting oneself from ticks					XPROMNTH	–0.019	0.043	0.66
		Visit 1 to visit 2	1.47	0.75, 2.91	0.26	V2*XPROMNTH	–0.002	0.042	0.97
		Visit 1 to visit 3	0.96	0.47, 1.99	0.92	V3*XPROMNTH	–0.078	0.089	0.38
XDAYS	Knowing the amount of attachment time needed for transmission of bacteria					XDAYS	0.019	0.059	0.75
		Visit 1 to visit 2	29.17	10.24, 83.07	<0.01	V2*XDAYS	0.010	0.065	0.88
		Visit 1 to visit 3	14.06	5.15, 38.41	<0.01	V3*XDAYS	0.076	0.077	0.32
XSMALL	Knowing the size of the ticks which cause the most disease					XSMALL	0.061	0.048	0.20
		Visit 1 to visit 2	0.69	0.38, 1.26	0.23	V2*XSMALL	–0.047	0.042	0.25
		Visit 1 to visit 3	0.52	0.28, 0.95	0.03	V3*XSMALL	–0.087	0.083	0.29
			Knowledge of and reported behaviors related to tick checks#						
CHCKSELF	Reporting checking oneself for ticks					CHCKSELF	0.006	0.049	0.91
		Visit 1 to visit 2	2.14	0.74, 6.15	0.16	V2*CHCKSELF	–0.078	0.096	0.42
		Visit 1 to visit 3	2.16	0.95, 4.91	0.07	V3*CHCKSELF	0.113	0.153	0.46
FNDTICK1	Reporting finding few ticks on oneself					FNDTICK1	0.054	0.044	0.22
		Visit 1 to visit 2	0.57	0.24, 1.39	0.22	V2*FINDTICK1	–0.004	0.054	0.94
		Visit 1 to visit 3	0.21	0.07, 0.65	0.01	V3*FINDTICK1	–0.156	0.098	0.11
FNDTICK2	Reporting finding many ticks on oneself					FNDTICK2	–0.036	0.039	0.35
		Visit 1 to visit 2	0.69	0.31, 1.50	0.35	V2*FNDTICK2	–0.010	0.038	0.79
		Visit 1 to visit 3	2.32	0.57, 9.41	0.24	V3*FNDTICK2	0.016	0.045	0.73

Table continues

DEET is used on the skin (decrease from visit 1 to visit 2), using a handheld mirror during tick checks (increase from visit 1 to visit 3), wearing protective clothing away from home (decrease from visit 1 to visit 2), and believing that DEET is safe for children (decrease from visit 1 to visit 2). The intervention was only associated with change in three of the six KAB variables associated with change in ARTCA levels. Overall, the data provided little evidence that change in the KAB measures was associated with change in ARTCA levels.

TABLE 4. Continued

KAB variable	Description of KAB variable	Model (change in KAB variable between visits)	Model with KAB variable used as the outcome			Model (change in ARTCA variable associated with change in KAB variable)	Model with ARTCA variable used as the outcome		
			Odds ratio	95% confidence interval	*p* value		β coefficient	Standard error	*p* value
TICKBITE	Reporting having had a tick bite					TICKBITE	0.011	0.043	0.81
		Visit 1 to visit 2	0.50	0.19, 1.27	0.15	V2*TICKBITE	–0.078	0.044	0.08
		Visit 1 to visit 3	0.37	0.10, 1.37	0.14	V3*TICKBITE	0.009	0.062	0.88
HCHKTCK	Reporting performing a tick check at home					HCHKTCK	–0.014	0.042	0.74
		Visit 1 to visit 2	3.18	1.59, 6.37	<0.01	V2*HCHKTCK	–0.076	0.051	0.14
		Visit 1 to visit 2	3.18	1.59, 6.37	<0.01	V2*HCHKTCK	–0.076	0.051	0.14
		Visit 1 to visit 3	1.94	0.99, 3.81	0.05	V3*HCHKTCK	0.046	0.114	0.69
ACHKTCK	Reporting performing a tick check away from home					ACHKTCK	0.033	0.064	0.61
		Visit 1 to visit 2	1.37	0.70, 2.67	0.35	V2*ACHKTCK	–0.128	0.064	0.04
		Visit 1 to visit 3	0.50	0.74, 3.05	0.26	V3*ACHKTCK	0.027	0.140	0.85
HANDHELD	Reporting use of a handheld mirror during a tick check					HANDHELD	–0.015	0.036	0.68
		Visit 1 to visit 2	8.86	3.60, 21.81	<0.01	V2*HANDHELD	0.035	0.043	0.41
		Visit 1 to visit 3	5.76	2.38, 13.96	<0.01	V3*HANDHELD	0.135	0.061	0.03
		*Knowledge of and reported use of tick repellents and acaricides***							
DEETSKIN	Knowing that DEET† is used on the skin					DEETSKIN	0.059	0.041	0.15
		Visit 1 to visit 2	5.41	2.95, 9.90	<0.01	V2*DEETSKIN	–0.092	0.046	0.05
		Visit 1 to visit 3	5.43	2.82, 10.47	<0.01	V3*DEETSKIN	–0.122	0.076	0.11
XDEETPCT	Knowing the minimum % of DEET needed to be effective					XDEETPCT	0.057	0.047	0.22
		Visit 1 to visit 2	97.67	16.29, 585.7	<0.01	V2*XDEETPCT	–0.057	0.057	0.31
		Visit 1 to visit 3	35.23	7.07, 175.6	<0.01	V3*XDEETPCT	–0.040	0.075	0.60

Table continues

DISCUSSION

To our knowledge, this was the first study to evaluate whether an educational intervention could change knowledge, attitudes, and behaviors regarding tick bites, tick bite prevention, and Lyme disease, and consequently whether changes in KAB were associated with changes in a biomarker of tick bites over time. While the intervention produced a difference between groups over time in terms of increased knowledge of ticks and Lyme disease, positive attitudes toward repellent use, and self-reported use of tick bite prevention methods, this change was not associated with change in ARTCA levels, a biomarker of longer-duration tick bites. The results indicate that more significant changes in KAB measures occurred between visit 1 (February–March) and visit 2 (June–July), the period when the majority

TABLE 4. Continued

KAB variable	Description of KAB variable	Model (change in KAB variable between visits)	Model with KAB variable used as the outcome			Model (change in ARTCA variable associated with change in KAB variable)	Model with ARTCA variable used as the outcome		
			Odds ratio	95% confidence interval	*p* value		β coefficient	Standard error	*p* value
SKINDEET	Reporting having used repellent containing DEET on the skin					SKINDEET	0.036	0.041	0.38
		Visit 1 to visit 2	6.07	2.74, 13.42	<0.01	V2*SKINDEET	–0049	0.042	0.24
		Visit 1 to visit 3	4.40	2.04, 9.48	<0.01	V3*SKINDEET	0.007	0.061	0.91
XPRMCLTH	Knowing that permethrin is used on clothing					XPRMCLTH	–0.023	0.039	0.56
		Visit 1 to visit 2	42.75	14.11, 129.5	<0.01	V2*XPRMCLTH	–0.013	0.041	0.75
		Visit 1 to visit 3	16.71	5.94, 47.03	<0.01	V3*XPRMCLTH	0.057	0.070	0.42
CLTHPERM	Reporting having used acaricide containing permethrin on clothing					CLTHPERM	0.047	0.043	0.28
		Visit 1 to visit 2	14.22	2.84, 71.21	<0.01	V2*CLTHPERM	–0.057	0.043	0.18
		Visit 1 to visit 3	11.76	2.40, 57.68	<0.01	V3*CLTHPERM	–0.004	0.058	0.95

* The table gives odds ratios and confidence intervals from logistic regression modeling using generalized estimating equations for associations with change in KAB variables (all dichotomous) over time and beta coefficients and standard errors from linear regression modeling using generalized estimating equations for associations of change in KAB variables with change in ARTCA levels (ng/μl) over time. ARTCA levels were log-transformed before modeling to better achieve a normal distribution. These models are presented side-by-side to allow the reader to see the change in KAB resulting from the intervention and whether this change was associated with a change in ARTCA level. Asterisks within variables indicate interaction terms.

† KAB, knowledge, attitudes, and behaviors; ARTCA, anti-recombinant tick calreticulin antibody; DEET, *N*,*N*-diethyl-*m*-toluamide.

‡ All KAB models also included INTERVENTION, VISIT 2, VISIT 3, INTERVENTION*V2, and INTERVENTION*V3. Results were controlled for sex, age, and race.

§ All ARTCA models also included KAB VARIABLE, VISIT 2, VISIT 3, KAB VARIABLE*V2, and KAB VARIABLE*V3. Results were controlled for sex, age, and race.

¶ The odds ratio represents the increase in odds of the behavior in the intervention group versus the comparison group between the given visit and visit 1.

\# Selected measures from a total of 17.

** Selected measures from a total of eight.

of repellent-related intervention materials were distributed to subjects and the period when people are most often engaged in outdoor activities. Strengths of the study included the relatively large sample size, the randomized assignment to groups, and the longitudinal evaluation.

The associations between the intervention and increases in knowledge and reported behaviors in the intervention group may be viewed as conservative because of the increases in knowledge and reporting of KAB measures in the comparison group over time. This increase displayed by the comparison group may be attributed to self-learning through the study instruments, as well as information sought through other sources because of interest in the topic. For example, since all subjects were requested to report their performance of tick checks and their use of repellents every 2 weeks on the checklists, many of the comparison group subjects might have adopted such behaviors because of these frequent reminders. The increase in KAB measures in this population due to this intervention may not be generalizable to the general population, since subjects in this study were motivated to participate in an intervention aimed at increasing the prevalence of preventive behaviors against tick bites.

The intervention effectively increased reported improved methodology in performing tick checks and familiarity with and use of DEET- and permethrin-containing products. Odds ratios indicating familiarity with DEET and permethrin products in the intervention group versus the comparison group were high across all visits. The belief that DEET is safe for adults increased significantly between groups across all clinic visits. Most prevalence studies of reported preventive behaviors against Lyme disease report low utilization rates for repellents (20, 36–38). Many scientific articles, as well as much of the health education literature distributed by health departments, promote use of insect repellent by adults (and in lower concentrations by children) as an effective method of preventing tick bites. The results of this study

TABLE 5. Associations of change in knowledge, attitudes, and reported behaviors regarding ticks and Lyme disease with changes in anti-recombinant tick calreticulin antibody levels over time for scaled KAB* variables, Tick Bite Prevention Project, Baltimore County, Maryland, 1999†

Scaled KAB variable‡	Description of KAB variable	Model (change in KAB variable between visits)§	Model with KAB variable used as the outcome			Model (change in ARTCA* variable associated with change in KAB variable)¶	Model with ARTCA variable used as the outcome		
			β coefficient	Standard error	*p* value		β coefficient	Standard error	*p* value
SHCLOTHE#	Reporting wearing protective clothing at home	INTERV	–0.122	0.286	0.67	SHCLOTHE	0.008	0.007	0.30
		V2*INTERV	0.592	0.273	0.03	V2*SHCLOTH	–0.010	0.007	0.18
		V3*INTERV	0.957	0.287	<0.01	V3*SHCLOTHE	0.011	0.013	0.38
SACLOTHE#	Reporting wearing protective clothing away from home	INTERV	–0.181	0.298	0.54	SACLOTHE	0.014	0.011	0.21
		V2*INTERV	0.594	0.329	0.07	V2*SACLOTHE	–0.024	0.011	0.03
		V3*INTERV	1.034	0.334	<0.01	V3*SACLOTHE	0.006	0.015	0.67
SHREPELL#	Reporting wearing repellent at home	INTERV	–0.550	0.263	0.04	SHREPELL	0.017	0.013	0.18
		V2*INTERV	1.396	0.357	<0.01	V2*SHREPELL	–0.016	0.015	0.27
		V3*INTERV	1.480	0.371	<0.01	V3*SHREPELL	0.016	0.017	0.35
SAREPELL#	Reporting wearing repellent away from home	INTERV	–0.725	0.254	<0.01	SAREPELL	0.003	0.016	0.21
		V2*INTERV	1.384	0.367	<0.01	V2*SAREPELL	–0.024	0.019	0.21
		V3*INTERV	1.488	0.361	<0.01	V3*SAREPELL	0.009	0.019	0.62
SSELEFF	Reported self-efficacy in protecting oneself from ticks	INTERV	0.798	0.466	0.09	SSELEFF	–0.005	0.005	0.40
		V2*INTERV	2.128	0.432	<0.01	V2*SSELEFF	0.001	0.005	0.77
		V3*INTERV	2.538	0.475	<0.01	V3*SSELEFF	0.003	0.005	0.51

* KAB, knowledge, attitudes, and behaviors; ARTCA, anti-recombinant tick calreticulin antibody.

† The table shows beta coefficients and standard errors from linear regression modeling using generalized estimating equations for associations of change in KAB variables (all continuous) with change in ARTCA levels (ng/μl) over time. ARTCA levels were log-transformed before modeling to better achieve a normal distribution. These models are presented side-by-side to allow the reader to see the change in KAB resulting from the intervention and whether this change was associated with a change in ARTCA level. Asterisks within variables indicate interaction terms.

‡ Selected measures from a total of nine.

§ All models also included INTERV (1 for intervention group, 0 for comparison group), VISIT 2, VISIT 3, INTERV*V2, and INTERV*V3. Results were controlled for sex, age, and race.

¶ All models also included KAB VARIABLE, VISIT 2, VISIT 3, KAB VARIABLE*V2, and KAB VARIABLE*V3. Results were controlled for sex, age, and race.

Factor analysis suggested use of a scale because of evidence of an underlying factor for the group of variables.

indicate that targeted, detailed information can increase knowledge of and positive attitudes toward repellent use and safety in an at-risk population of adults.

The comparison group had evidence of knowledge and behaviors related to tick bite prevention at baseline and an increase in KAB measures over the intervention period. We thus believed that the most valid approach to statistical analysis was to model first whether the intervention was associated with a change in KAB measures and next whether the change in KAB measures was associated with a change in ARTCA levels, rather than to model change in ARTCA levels directly in the intervention and comparison groups. This method had the advantage of directly assessing what we were interested in while helping us avoid the potential to inappropriately conclude that the intervention was not effective when significant cross-contamination was the explanation.

ARTCA levels were low at baseline, and only small changes were observed at follow-up visits. Few subjects evidenced increases that would suggest longer-duration tick bites during the study. Several factors may have influenced the absence of significant changes in ARTCA levels throughout the study. Tick activity may have been low during the study period. The summer of 1999 in Baltimore County was unusually hot and dry, and anecdotal reports suggested low tick activity. Study subjects rarely reported tick bites or even finding ticks on their skin or clothing. Consequently, a lack of change in ARTCA levels could have been due to an absence of tick bites among study subjects. Furthermore, for us to have observed increases in ARTCA levels over time, subjects would have had to have tick bites of at least 3 days' duration. Since all subjects were receiving bimonthly checklists which requested information on performance of tick checks and repellent use, it is possible that subjects were preventing tick bites, especially bites of longer duration. In planning the study, we performed power calculations using population values for ARTCA levels and variability from prior studies from other geographic areas. These calculations suggested that the study would have the power to detect a very small decline in ARTCA levels if such a change existed. However, ARTCA levels in our study population in Baltimore County were much lower and much less variable than we expected. We thus believe that we had limited power to detect changes in ARTCA levels.

Blood was obtained only three times during the study, with approximately 9–11 weeks between collections. Prior studies suggested that ARTCA levels may increase and subsequently decline in as little as 45 days in rabbits, which suggests that changes in ARTCA levels could have been missed between clinic visits (27). In humans, studies of the kinetics of ARTCA and anti-tick saliva antibodies suggest that levels increase and decrease within a few weeks after a person receives a tick bite. A study of recent tick bite recipients found that ARTCA levels increased within 19–26 days and remained elevated for approximately 50 days (28). However, a study of outdoor workers in New Jersey found that anti-tick saliva antibody levels had declined significantly by 3 months after probable tick bites (19).

These results indicate that a tailored intervention increased knowledge, attitudes, and behaviors related to tick bite prevention in a population living in an area with endemic Lyme disease. However, this change did not correspond to a change in ARTCA levels. The utility of ARTCA as a biomarker in this type of intervention study is yet to be determined, and future research may benefit by utilizing ARTCA in areas with a higher incidence of tick bites. Furthermore, proteins injected within the first hour of a tick bite may be explored as indicators for shorter-duration bites. Data suggest that other tick-borne diseases are transmitted within a shorter period during a tick bite (8).

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