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Assessment Of Foodnet Population Survey Respondents As A Control Group To Determine Stec Risk Factors

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Assessment of FoodNet population survey respondents as a control group to determine STEC risk factors

M.P.H. Thesis

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Introduction: Shiga toxin-producing *Escherichia coli* (STEC)are important foodborne pathogens, causing approximately 30 deaths a year in the US. Case-control studies to monitor risk factors are challenging; finding representative controls can be labor-intensive. The FoodNet Population Survey (FNPS) is conducted periodically and asks about foodborne disease risk factors.

Objectives: To determine leading risk factors for STEC in Connecticut using the 2006 FNPS participant responses as controls.

Methods: Cases were reported STEC cases in Connecticut 2000-2009 who were interviewed for risk factors following onset. Controls were respondents to the Connecticut portion of the 2006 FNPS. Comparable questions for exposure to dietary, travel and recreational risk factors in the 7 days before illness onset (cases) or interview (controls) were included. FNPS questions that were non-identical to case questions were examined when similar composite variables could be created.

Results: Data from 559 cases and 1,801 controls on 14 variables were included. Statistically significant risk factors, stratified by age, included 'ground beef' for the 6-17 year and ≥18 age groups (OR 2.24, 95% C.I 1.47-3.43; OR 1.8, 95% C.I. 1.35-2.43, respectively), 'pink burger patties' in the 6-17 year group (OR 2.07, 95% C.I. 1.01-4.25), and for all age groups, 'traveling outside of the U.S.' (age-adjusted OR 6.66, 95%C.I. 3.36-13.19) and 'visiting a petting zoo' (age-adjusted OR 4.06; 95% C.I. 3.28-7.92). Among similar but not identical variables, poultry was a risk factor for adults while consumption of lettuce, spinach and sprouts were risk-reducing factors in various age categories.

Conclusions: Several known STEC risk factors (ground beef, pink burger, travel, petting zoo) were confirmed, but several other findings are suspect. FNPS participants' responses show potential for use as controls but FNPS questions need to be matched to those asked cases.

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I. Introduction

Shiga toxin-producing *Escherichia coli*, including O157:H7 and non-O157 serogroups are major pathogens that have been identified in outbreaks as well as sporadic cases of foodborne illness (Johnson et al, 2006). The widespread risk for foodborne diseases has prompted active surveillance for a number of pathogens, including STEC serogroups. Since 1995, the Foodborne Diseases Active Surveillance Network (FoodNet) has enabled a collaborative surveillance effort between the Centers for Disease Control and Prevention, the U.S. Department of Agriculture's Food Safety and Inspective Service (USDA-FSIS), the Food and Drug Administration (FDA) and 10 state health departments, including Connecticut (Bender et al, 2004). An integral component within the FoodNet program, the FoodNet Population Survey (FNPS) helps to enable better estimation of the true burden of diarrheal diseases in the population (Voetsch et al, 2007).

The case-control study design is one of the most commonly used approaches in epidemiological studies, as it can be conducted in less time and with fewer resources than a traditional prospective cohort (Schulz and Grimes, 2002). However, the process involved in acquiring a representative control sample is cumbersome, time-consuming and susceptible to bias (Schulz and Grimes, 2002). In designing a case-control study to examine leading STEC risk factors and monitor changes in them over time, the *E. coli* O157 and Other Shiga Toxin-Producing *E. coli* Questionnaire from the Connecticut Emerging Infections Program (EIP) data provides detailed information on exposures to diverse dietary, recreational and travel factors previously associated with illness (CDC, 2012). Considering possible control groups, the FoodNet Population Survey stands out as a readily available and comprehensive database on important risk factors, which should be analyzed for its potential as a representative population-based control group (Schulz and Grimes, 2002). The following study employs these two resources in evaluating leading risk factor trends for STEC illness in the state of Connecticut, from 2000 to 2009. Simultaneously, it discusses advantages and disadvantages of using the 2006 FNPS as a practical source of population-based controls, suggesting possible modifications that might improve its suitability in the future. The FNPS is currently undergoing revision prior to planned implementation in 2015-2016.

II. Background

The burden of STEC

Shiga toxin-producing *Escherichia coli* are agents responsible for 10% of approximately 9 million cases of foodborne illness observed in the US each year (Scallan et al, 2011). Of the ~250 STEC serogroups found to produce Shiga toxin, over 100 groups have been associated with sporadic cases and outbreaks of disease (Gould et al, 2009). Evidence on the pathogenicity of the O157:H7 serogroup is abundant, and it is currently identified as the leading cause of HUS in the United States (Gyles, 2007). Also, findings on the increasing incidence of various non-O157 serogroups have led to the development and implementation of more accurate testing for better identification of non-O157 STEC, as well as improved surveillance to better define their public health importance and significant risk factors (Hughes et al, 2006).

A potent pathogen, only a low infectious dose is needed to produce an STEC infection, with clinical manifestations appearing 3-4 days after exposure (Boyer and Niaudet, 2011). Most commonly, exposed individuals experience abdominal pain, fever, vomiting and watery diarrhea (Gyles, 2007). Bloody diarrhea occurs in about 90% of all cases of STEC infections (Gyles, 2007). Most individuals who do not experience complications leading to HUS and who receive sufficient rehydration therapy will experience full recovery (Razzaq, 2006). However, the production of Shiga toxin, hallmark characteristic of the STEC group, results in various degrees of illness, from milder diarrheal disease to HUS (Hale et al, 2012). HUS most often presents as a combination of thrombocytopenia, hemolytic anemia and acute renal failure (Gianantonio et al, 1964). It is estimated that between 5 and 15% of cases develop HUS, with young children, the elderly and immunocompromised persons having higher risk of severe illness (Gianantonio et al, 1964).

In spite of the commonly positive prognosis for recovery for STEC cases, the social and financial burden of illness associated with these organisms is an important health problem. Estimates in 2012 suggest that STEC O157 and non-O157 resulted in over 265,000 cases of disease and 30 deaths in the US (Scallan, 2011). The cost per case of illness with STEC O157 is estimated at $10,446 (Marks et al, 2013). For non-O157 cases, determining exact costs is complicated and possibly underestimated and inadequate data is available on the number of deaths attributed to the disease (Marks et al, 2013). However, it has been calculated that over $51 million a year are spent in the treatment of non-O157 diseases, based on estimates between 2005 and 2010 (Marks et al 2013).

Risk factors for STEC

STEC are foodborne and waterborne pathogens that can be transmitted to humans through the fecal-oral route, by the consumption of contaminated water and food products (Kaspar et al, 2010). Although various species of domestic and wild ruminants have been identified as reservoirs for STEC, beef cattle are considered a primary source of infection (Hussein & Bollinger, 2005). A 2011 study looking at STEC serogroups in ground beef across the United States found that the prevalence of contamination could be as high as 24.3%, with isolation of the organism confirmed in 7.3% of 4,133 samples (Bosilevac 2011) Large outbreaks in both Washington State and Minnesota have also been linked to ground beef consumption, with isolates found in raw/undercooked patties as well as cooked and frozen burgers (Bell et al, 1994 and Belongia et al, 1991). Consumption of unpasteurized or raw dairy products, such as milk and cheeses, has also been linked to infection and disease (Baylis, 2009). In a Portland outbreak, most infections were tracked to the consumption of raw milk from a common dairy provider contaminated with STEC (Keene et al, 1997).

Besides animal products, several vegetables have also been implicated in outbreaks of diseases due to STEC serotypes. A large outbreak of non-O157 STEC that started Germany in the Spring of 2011 and affected individuals in 16 countries resulted in over 4,000 cases of disease, a 23% rate of HUS among mostly adult cases and 54 deaths (Frank et al, 2011). In the US, a 2014 outbreak of STEC caused by the consumption of raw clover sprouts resulted in 19 cases in 6 states (MMWR, December 2005). Various studies have also looked at STEC isolates found in lettuce, which is most often consumed raw (Mazaheri 2014) In the United States, a 2011 outbreak involving 58 cases in 10 states traced the infection to romaine lettuce contaminated with O157:H7 supplied to various stores from a single farm provider (Slayton 2013).

Several recreational activities involving exposure to contaminated water sources and colonized animals have been associated with cases of disease. Between 2004 and 2005, 3 outbreaks in North Carolina, Florida and Arizona involving over 100 cases were reported; most cases involved children who had visited petting zoos at agricultural fairs and festivals (MMWR, CDC 2005). Studies screening petting zoo animals have found isolates of both STEC and EHEC (Enterohemorrhagic *E. coli*) in both cows and goats at petting zoos and farms (DebRoy and

Roberts, 2006). Contaminated water with exposure either by direct consumption and/or contact through swimming has also been involved in STEC outbreaks (Mathusa, 2010). A 1991 outbreak where cases presented hemorrhagic colitis was associated with swimming at a lake, suspected to be contaminated with fecal matter (Keene, 1994). FoodNet surveillance between 2004 and 2009 also found that 13% of cases were associated with recent travel to common destinations outside of the US, including Mexico and India (Kendall et al, 2012).

FoodNet and the Emerging Infections Program

Established in 1996, the Foodborne Diseases Active Surveillance Network, known as FoodNet, has become the primary component of the Center for Disease Control and Prevention's Emerging Infections Program (Scallan, 2007). As a multiagency collaborative effort, this program performs an essential role on food safety through population-based active surveillance of laboratory-confirmed cases of foodborne pathogens (Steinmuller et al, 2006). Currently, a network of 10 state health departments (California, Colorado, Connecticut, Georgia, Maryland, Minnesota, New Mexico, Oregon, Tennessee, and New York) actively partakes in this program (Scallan, 2007). As the leading component to monitor foodborne illnesses in the CDC's Emerging Infections Program (EIP), FoodNet is tasked to accurately determine the burden of foodborne disease, what risk factors and in which proportions exposure is associated with disease and how to effectively and promptly identify, investigate and control outbreaks (CDC MMWR, 1997).

As of 2011, FoodNet sites covered 48 million persons in the US, or 15% of the total population, through active surveillance of laboratory-confirmed cases of 9 major foodborne pathogens, including STEC (Scallan et al, 2011). The 2014 MMWR Report for Incidence and

Trend of Infection with Pathogens Transmitted Commonly Through Food, the product of FoodNet's surveillance through 2013 reported over 19,000 hospitalizations and 80 deaths (CDC MMWR, 2014). These annual reports since the establishment of FoodNet provide useful information on characteristics of cases, major risk factors, and yearly outbreaks. These reports are also essential in determining changing trends of disease since the establishment of FoodNet. The 2014 report noted a decreasing trend in overall foodborne infection rates and also provided suggestions as to how to increase regulatory measures of the food industry and better educate the public on foodborne illnesses (CDC MMWR, 2014).

Active surveillance of cases, hospitalizations and deaths from foodborne disease is a useful, but incomplete strategy to assess the true national burden of disease (Cantwell et al, 2010). The FoodNet Population Survey, an adaptation of the standard Behavioral Risk Factor Surveillance System (BRFSS) method, was developed as a tool to address the impact of cases that go undiagnosed, and therefore are not reported (CDC FoodNet, 2012). Population-based surveys using stratified sample random digit dialing are performed; prevalence of diarrheal illnesses and exposure to known risk factors, including foods, water sources, recreational activities and travel are determined (CDC FoodNet, 2012). Since 1996, five surveys have been conducted throughout FoodNet sites, most recently in 2006-2007. This latest version included the largest catchment population (46 million persons) at all 10 FoodNet sites (CDC FoodNet, 2012). Another survey is planned for 2015-2016. The surveyed area is being expanded and the survey instrument is being revised, making this a timely study.

III. Methods

Study design, case definition and ascertainment

For this unmatched case-control study, cases were all Connecticut residents reported to FoodNet who had laboratory-confirmed samples with Shiga toxin-producing *Escherichia coli* and who completed the Connecticut Emerging Infections Program's *E. coli* O157 and other Shiga toxin-producing *E.coli* Questionnaire between 2000 and 2009. During this period, the Connecticut catchment area included the entire state (~3.5 million population). FoodNet staff actively surveyed cases through laboratories to determine all confirmed STEC cases, and performed phone interviews using the STEC questionnaire for additional information on clinical manifestations of disease and food, recreational and travel-related risk factors. .

Population-based controls

The 2006 FoodNet Population Survey database was selected as a source for population-based controls. Controls were defined as all survey respondents within the Connecticut catchment area who completed the questionnaire through telephone interviews between May 15th 2006 and May 23rd, 2007. For the study, 1,801 respondents to the 2006 survey were extracted from a general database including all 10 FoodNet sites. Sample selection at FoodNet sites was performed through disproportionate stratified sample random-digit-dialing (CDC, 2012).

Questionnaires

The Connecticut Emerging Infections Program implemented the *E. coli* O157 and Other Shiga toxin-producing *E.coli* questionnaire as instrument to ascertain risk factors in laboratory-confirmed STEC cases in Connecticut. Although this questionnaire has been revised throughout the years, each version captures major risk factors for sporadic infection based on yes/no responses. The 2008 version of the questionnaire, used as reference in this study, included both open and close-ended questions, depending on the type of infection (outbreak versus sporadic case). A detailed food history included various sections, grouped by food types. Recreational and drinking water, travel and farm/animal exposure questions were also included. All variables considered were based on exposure seven days prior to the onset of illness.

The FoodNet Population Survey Adolescent, Adult and Pediatric Questionnaire 2006-2007 was the primary tool for the assessment of exposure and data collection for controls. The survey was conducted between May 2006 and April 2007, including all 10 FoodNet sites (CDC, 2012). The questionnaire included two food exposure sections with yes/no questions, divided in modules based on food types. Food practice and beliefs, animal contact, prion disease, drinking and recreational water sections to determine exposure factors were also included. A community section with questions regarding place of residence and demographic information of the respondent/child of the respondent was available. Lastly, questions pertaining to risks in each state provided site-specific exposure information.

Statistical analysis

Epi info 7 was employed in the calculation of all frequency values for risk factors in both cases and controls. Epi Info StatCalc was used to generate 2x2 tables for the calculation of crude and adjusted odds ratios using stratified analysis and the Mantel-Haenzel adjusted OR and Chi-square. To obtain risk factor frequencies for cases and controls, each group was stratified by age (0-5, 6-17, and 18 years and older), sex, season (December-February, March-May and October-November, June-September) and in cases, additional stratification was performed to account for presence of the O157 serogroup. All variables in the FNPS with identical or comparable questioning were considered for analysis. Several variables found in the EIP STEC questionnaire but missing questions and/or responses in the FoodNet Population Survey (living in a farm, attending a childcare setting and employment involving handling food) were discarded.

Composite variables for statistical analysis

Questions in the EIP STEC Questionnaire and FoodNet Population Survey were carefully reviewed to determine congruency. All variables included in the final analysis were based on exposures in the 7 days prior to disease onset. Recreational variables based on exposures in the month prior to disease onset were excluded from analysis. All questions considered identical or nearly identical were included in the analysis without additional adjustment. A number of relevant but non-identical questions were found on both surveys; these questions were most often posed in the EIP STEC survey as single, all-encompassing measures of exposure, and divided into various, more specific questions in the Population Survey. In the case of such non-identical variables, composite variables were generated. Composite variables consisted of 2 or more questions from either the EIP STEC Questionnaire or FoodNet Population Survey.

Composite variables for controls were formulated through the Recode command, using Epi Info 7 (CDC Epi Info Tutorials, 2015). Two types of composite variables were created for the following analysis. The variables ‘ground beef’, ‘poultry’, ‘lettuce’, ‘spinach’ and ‘sprouts’, 5 of 12 dietary variables, were created through composite variables that counted any affirmative response (“yes” to exposure question) as an exposure. Negative responses were only counted as true non-exposure when all answers within the composite variable were negative. Missing values were counted as “unknown”. In cases where variables contained missing values for one question, but affirmative values for another question in the composite variable, the respondent was classified as "exposed." Otherwise, respondents with all missing values, or missing and negative values only, were classified as "unknown" and removed from the analysis (Appendix I).

Exposures 'ground beef', 'pink burger' and 'raw cider' were composed of duplicate responses to the same question. After analysis to verify that duplicate questions were identical, composite variables were generated using the Recode command in Epi Info 7 (CDC Epi Info Tutorials, 2015). Assessment of duplicate variables showed that these complemented missing responses from the first version of the question. Therefore, duplicate composite variables assigned exposure value based on the one available response. For exposed respondents, the presence of an affirmative response was recorded as an exposure; participants with negative responses were classified as unexposed. Careful review of these variables showed no overlap of answers: duplicate variables for each exposure contained responses to missing values in the original question, but no duplicate responses.

IV. Results

Demographic characteristics of cases and controls

Demographic characteristics for cases are provided in Table 1. A total of 559 cases were included in the analysis. All cases were respondents to the STEC questionnaire between February 25th, 2000 and December 23rd, 2009. Age of respondents ranged from 0 to 93 years old, with a median age of 15 years. Age distribution for cases was 18% 0 to 5 years, 40% 6 to 17 years, and 42% 18 and older. Racial distribution of cases showed a majority of non-Hispanic white participants (89%), followed by Hispanic (6%), black (2%) and other races (2%). Female cases were predominant compared to males (57% and 43%, respectively). Laboratory confirmation of infection by serogroup identified STEC O157 as the most common agent, found in 69% of cases, compared to all non-O157 serogroups, representing 31% of overall cases.

Table 1 also includes demographic characteristics of the control group. The 1,801 respondents in the control group ranged from 1 to 99 years old, with a median age of 52 years. Age distributions for participants in the population survey were notably different from that of cases: 5% 0 to 5 years, 10% 6 to 17 years, and 86% 18 years and older. Distribution by race and sex were the same as those for cases.

Odd ratios for exposure variables

Crude odd ratios were calculated for 14 dietary, travel and recreational variables with identical questions or using composite variables. When stratified by sex, consumption of ground beef and beef patties was significant for both males and females (OR 2.27, 95% C.I. 1.65-3.2; OR 1.53, 95% C.I. 1.17-1.99, respectively) (Tables 2a and 2b). Odd ratios for foreign travel were also found to be statistically significant in males and females (OR 5.11, 95% C.I. 2.34-11.19; OR 7.98, 95% C.I. 3.71-173), as was visiting a petting zoo (OR 3.17, 95% C.I. 1.66-6.06; OR 7.41, 95% C.I. 4.0-12.72, respectively). In males, consumption of raw/pink burger patties was weakly associated with risk of infection, but this value was not statistically significant. For females, consumption of poultry was strongly associated with an elevated risk for STEC infection (OR 2.23, 95% C.I. 1.39-3.57).

Several variables found statistically significant in males and females were negatively associated with infection risk (Tables 2a and 2b). Lettuce consumption in both males and females presented odd ratios below 1 (OR 0.23, 95% C.I. 0.16-0.35; OR 0.28, 95% C.I. 0.2-0.41, respectively). Spinach and sprouts also showed an inverse association to risk, with statistically significant values in both gender categories (males: spinach OR 0.37, 95% C.I 0.21-0.65 and sprouts OR 0.04, 95% C.I. 0.01-0.12; females: spinach OR 0.66, 95% C.I. 0.46-0.95 and sprouts OR 0.06, 95% C.I. 0.02-0.13). Drinking raw or unpasteurized apple cider was inversely associated with increased risk in males (OR 0.31, 95% C.I. 0.11-0.87), but not in females.

When stratified by age groups, visiting a petting zoo was a statistically significant risk factor in all categories (0-5 years: OR 7.80, 95% C.I. 1.75-34.71; 6-17 years: OR 4.47, 95% C.I. 1.32-15.15; 18 years and older: OR 3.12, 95% C.I. 1.68-5.79) (Tables 3a-3c). Foreign travel also showed a strong association with increased infection risk in all age categories (0-5 years: OR undefined, lower 95% CI 1.10; 6-17 years: OR 8.1, 95% C.I 1.85-35.44; 18 years and older: OR 5.56, 95% C.I. 2.74-11.25). Consumption of ground beef patties was also significantly associated with risk for the two older age strata (6-17 years: OR 2.24, 95% C.I. 1.47-3.43; 18 years and older: OR 1.8, 95% C.I. 1.35-2.43). Ingestion of pink burger meat was associated with elevated risk of disease in the 6-17 years category (OR 2.07, 95% C.I. 1.01-4.25), while eating poultry (OR 1.77, 95% C.I. 1.1-2.83) and salami (OR 2.99, 95% C.I. 2.04-4.37) were significant risk factors, but only for respondents ages 18 and older.

Inverse associations in several variables were also observed in age strata (Tables 3a-3c). Both lettuce (6-17 years: OR 0.56, 95% C.I. 0.33-0.97; 18 years and older: OR 0.35, 95% C.I. 0.23-0.53) and sprouts (6-17 years: OR 0.02, 95% C.I. 0.003-0.15; 18 years and older: OR 0.1, 95% C.I.0.05-0.21) were statistically significant factors inversely associated with increased risk. Finally, eating spinach was also associated with decreased risk for infection, but only for the 6-17 years group (OR 0.32, 95% C.I. 0.15-0.69).

In order to determine variability in risk factors by seasons, risk factors were also stratified by three seasonal categories: summer (June-September), winter (December-February) and spring/fall (March-May and November-December) (Tables 4a-4c). Stratified by seasonality, foreign travel remained a significant risk factor in all of the categories (summer OR 4.8, 95% C.I. 1.97-11.7; winter OR 36.9, 95% C.I. 6.57-207.25; spring/fall OR 6.4, 95% C.I. 2.97-13.79). Similarly, visiting a petting zoo was statistically significant throughout the three seasons (summer OR 2.85, 95% C.I. 1.61-5.04; winter OR 6.07, 95% C.I. 1.19-31.12; spring/fall OR 7.05, 95% C.I. 3.19-15.5). Ground beef was found to be a significant risk factor for infection for the summer (OR 1.95, 95% C.I. 1.45-2.62) and spring/fall months (OR 2.68, 95% C.I. 1.37-5.23). Ingestion of poultry was also associated with risk for disease in the summer (OR 1.69, 95% C.I. 1.01-2.82) and winter (OR 4.85, 95% C.I. 1.13-20.77). Consumption of undercooked (pink) beef patties was significantly associated with risk of infection by season, but this association was restricted to summer months (OR 1.56, 95% C.I. 1.02-2.39).

Seasonal odd ratios included several inverse associations between exposure and risk (Tables 4a-4c). Eating lettuce remained statistically significant across all seasons (summer OR 0.27, 95% C.I. 0.18-0.4; winter OR 0.32, 95% C.I. 0.16-0.65; spring/fall OR 0.22, 95% C.I. 0.14-0.35). In the case of spinach, the association remained significant only in the summer (OR 0.44, 95% C.I. 0.28-0.7) and the winter (OR 0.29, 95% C.I. 0.1-0.84); inverse associations with consumption of sprouts were significant for summer (OR 0.05, 95% C.I. 0.02-0.12) and spring/fall months (OR 0.04, 95% C.I. 0.009-0.15). Lastly, drinking unpasteurized/raw apple cider also presented an inverse association with risk, but this values only remained significant in the summer category (OR 0.36, 95% C.I. 0.14-0.94).

Visiting a petting zoo 7 days prior to onset of illness for cases and the survey interview for controls was selected for additional analysis as the variable had been a statistically significant risk factor across all classifications and categories (Table 4a-4c). Stratified analysis of age groups by seasons resulted in significant values in the spring/fall and summer categories, but different age groups in each season. For the summer, only the 6-17 years group remained statistically significant (OR 5.19, 95% C.I. 0.67-40.32), while the odds ratio in the 18 years and older group maintained significance during the spring/fall months (OR 5.8, 95% C.I. 2.09-16.08). In the winter, the 0-5 year group presented a high odds ratio of 6.8, but this value was not statistically significant.

Adjusted, age-stratified analysis for visiting a petting zoo was strongly associated with risk of STEC infection for all age groups (Mantel-Haenzel OR 4.06, 95% C.I. 2.42-6.83). To get a composite analysis for foreign travel, adjusted, age-stratified analysis was also performed (Table 5). Foreign travel was similarly associated with high risk of STEC infection for all age groups (OR 6.66, 95% C.I. 3.36-13.19) (Table 5).

To further understand the effect of certain statistically significant dietary associations, we performed a stratified analysis of ‘poultry’, ‘lettuce’, and ‘spinach’ based on ground beef consumption. Table 6 presents the odds ratio of consumption of poultry stratified by eating ground beef in respondents 18 years and older. This analysis showed a significant increase in risk in ground beef and poultry consumers (OR 2.14, 95% C.I. 1.17-3.92), but not in poultry consumers who did not consume ground beef.

When lettuce consumption in all three age groups was stratified by ground beef, an inverse correlation to risk of illness remained in both strata in all age categories (Table 7). Lastly, odds for spinach consumption stratified by ground beef were calculated for all age categories. For most categories, stratification resulted in no measurable effects. For the 6 to 17 years category, a statistically significant effect in ground beef consumers remained after stratification (Table 8).

Discussion

The establishment of The Foodborne Diseases Active Surveillance Network has been a pivotal step in the improvement of active surveillance for foodborne illness in the United States, providing representative, population-based data to detect the burden of disease, and target interventions towards its improvement (Scallan, 2007). Our results provide insight into the potential usefulness of the most recent (2006-2007) version of the Population Survey as a resource for control data. This information can be used to help develop the next Population Survey iteration scheduled to be implemented in the next year. Next, we elaborate on the effect of discrepancies between the STEC questionnaire and FoodNet Population Survey, its impact on these results, and possibilities for improvement.

The demographic characteristics for cases and controls in this study clearly highlight significant age differences between these groups (Table 1). In both the 0 to 5 age and 6 to 17 year age categories, cases had almost four times the percentages of respondents compared to controls, resulting in fewer controls than cases in each. The small size of these control groups made it difficult to further stratify on potentially confounding factors. To take full advantage of the STEC case distribution by age and the fact that the majority of cases are in children, a larger representation of children among population survey respondents is needed (Kassenborg et al, 2004).

The consumption of ground beef and raw/pink beef has been previously associated with onset of STEC cases (Hadler et al, 2011). In this study, ground beef, defined as the consumption of any frozen or fresh beef in hamburger patties and other dishes, was a consistent risk factor across most categories, except in the winter, when stratified by season. Previous research on the seasonality of ground beef as a risk factor for STEC have found higher prevalence rates in the warmer months of the year, consistent with the results presented here (Hussein and Bollinger, 2005).

In addition to ground beef, we analyzed a closely related exposure, based on the consumption of fresh and/or previously frozen undercooked or raw beef 7 days prior to disease onset (cases) or interview (controls). The consumption of raw hamburger has also been associated with sporadic cases of STEC O157 and non-O157 (Kassenborg et al, 2004). Our results indeed suggest that when stratified by sex and age, consumption of pink burger appears to be a risk factor; however, these results were not consistently significant across all age categories, suggesting that a larger sample size might be needed in determining the full impact of this factor.

Throughout our analysis, travel outside of the US was a strong risk factor for the development of illness due to STEC. Foreign travel has become an important surveillance component for foodborne pathogens, as it is estimated that approximately 40% of travelers to foreign countries will experience diarrheal illness (Kendall et al, 2012). Our results support the elevated risk of exposure to STEC during travel abroad as this was a risk factor for all age groups, with its highest OR value in the winter, when stratified by season (OR 36.9, 95% C.I. 6.57-207.25). Although information regarding travel destinations was not available for further analysis, these results suggest that seasonality of travel risk is of interest.

Attending farms/petting zoos has been a demonstrated risk factor for foodborne illnesses, especially for diseases caused by agents such as STEC that have ruminants as reservoirs, as direct contact with animals and fecal matter poses a higher risk of infection (Weese et al, 2007). Our analysis suggests that petting zoo visits were important factors for disease across all age categories and seasons. As expected, the higher odd ratios were associated with youngest age groups, with children 0 to5 having the greatest overall point estimate of risk based on this variable (OR 7.8, 95% C.I. 1.75-34.71). Age-adjusted analysis using the Mantel-Haenzel method supported the crude OR values, with petting zoo remaining a highly significant risk factor after adjustment.

Spinach and lettuce have been implicated in outbreaks of STEC (Grant et al, 2008 and Slayton et al, 2013). However, throughout our analysis, inverse associations between eating lettuce or spinach and disease were found across all ages and seasonal categories. In order to address these discrepancies, we performed additional analysis stratifying these variables, as well as poultry, by consumption of ground beef, the leading dietary risk factor in our study. The results from such analysis were mixed (Tables 6-8). Poultry consumption, once stratified by the 'ground beef' variable, was only a significant risk factor in consumers of both meat categories.

In the case of lettuce, adjusting for ground beef did not account for this correlation, as the risk-reduction effect remained statistically significant in most categories. Conversely, stratification by ground beef consumption for spinach resulted in mostly non-significant results, except for the ground beef and spinach consumer category, where the reduced effect remained post-adjustment. It is important to take into consideration that 'lettuce' and 'poultry' variables for the controls were the result of multiple questions recoded as a composite variable of a single exposure. We predict that further analysis into these variables might in fact account for part of the effect observed, as our estimation of factors to add to these variables might not be representative of what cases could recall from simpler and broader questions.

Using the 2006 FNPS and 2000-2009 FoodNet data on STEC reported cases has several advantages. All datasets used in this study were collected and organized prior to analysis, eliminating the obstacle of surveying for respondents in either group. In particular, data on all cases can be used as nearly all cases are initially interviewed as part of surveillance. Also, both surveys utilized a 7-day recall period, which allowed for responses to be comparable in terms of exposure time. The Population Survey and the STEC questionnaire were also effective in that they both included questions regarding important risk factor for Shiga toxin-producing *E. coli* infections. For questions that did not appear completely comparable, the ease of questioning in both surveys made it possible to generate alternative variables for controls, more like those asked cases. Although we did not take advantage of it, the fact that the FNPS collects ZIP code of respondents enables the potential of matching with cases on ZIP code socioeconomic status if not actual ZIP code.

Several limitations hindered our current analysis. As previously mentioned, the differences in age distribution between cases and controls, especially for the 0 to 5 years age group impacted the power to detect differences for those age groups, especially on low prevalence variables. Also, recoding variables from the 2006 FNPS into composite variables might have potentially generated dissimilar comparison groups, as questions from the STEC questionnaire were much broader. It is unknown if such differences would have affected recall in cases, controls or both respondent groups (Cantwell et al, 2010).

With the FoodNet Population Survey currently under revision, the opportunity of adapting this tool into a more effective national databank for controls to be used in studies of foodborne disease is in sight. In our study, we found that the 2007 version of the FNPS, although accessible and clear, presented major challenges as a useful control group. However, modifications to FNPS to achieve better comparability to questionnaires for reported cases might greatly expand its usefulness. A major difference between the 2007 FNPS and FoodNet STEC surveys involves the use of general, one-question approach per risk factor in the STEC questionnaire, and the more specific questioning used for the FNPS. The standardization of questions among questionnaires, to include sections that are comparable, with questions that clearly describe all possible exposure within each category would be a useful change in adapting the survey for multiple purposes.

V. Conclusion

The findings of this study suggest that the 2007 FoodNet Population Survey has the potential, pending modifications, to be a useful tool in case-control studies to examine the prevalence of foodborne illness. Mixed results, some which resemble available literature, and some which suggest that our current methodology may have biased our results, generated unexpected effects. Still, a number of well-studied risk factors remained significant across all analysis categories, which suggests that this approach might still be able to detect some true associations. With the FNPS undergoing revisions, we recommend changes be made that allow this instrument to be better adapted as a databank for study controls, which are often cumbersome to obtain. Through the application of these improvements, we predict that a new version of the FoodNet Population Survey has the potential to become a versatile tool of epidemiologic research.

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VII. Tables

Table 1. Demographic characteristics of cases and controls

		Cases	559	Controls	1801
Characteristic		n	%	n	%
Age (years)					
	0 to 5	103	0.18	85	0.05*
	6 to 17	222	0.4	174	0.1*
	≥18	234	0.42	1542	0.86*
	Total	559		1801	
Race					
	Non-Hispanic white	467	0.84	1493	0.83
	Non-Hispanic black	12	0.02	43	0.02
	Hispanic	34	0.06	104	0.06
	Other/Unk	46	0.08	161	0.09
	Total	559		1801	
Sex					
	Female	321	0.57	1066	0.59
	Males	238	0.43	735	0.41
	Total	559		1801	
Serotype					
	O157	375	0.67		
	non-O157	170	0.30		
	Unknown	14	0.03		
	Total	559			

* p<0.001 cases compared to controls. All other comparisons were non-significant

Table 2a. Prevalence and odds for exposure in preceding 7 days between cases and controls in males

	Cases			Controls					
Variables	N	Yes	%	N	Yes	%	OR	95% C.I.	p-value
Ground beef	234	166	0.71	724	375	0.52	2.27	(1.65, 3.12)	<0.0001
Pink burger patties	156	55	0.35	227	60	0.26	1.52	(0.97,2.36)	0.06
Poultry	205	179	0.87	356	295	0.83	1.42	(0.87,2.34)	0.16
Salami	210	59	0.28	356	123	0.35	0.74	(0.51,1.07)	0.11
Jerky	212	9	0.04	356	26	0.07	0.56	(0.26,1.23)	0.14
Venison	211	1	0.005	359	11	0.03	0.15	(0.02,1.18)	0.04
Lettuce	203	113	0.56	363	306	0.84	0.23	(0.16,0.35)	<0.0001
Spinach	203	17	0.08	365	72	0.2	0.37	(0.21,0.65)	0.0003
Sprouts	207	3	0.01	376	103	0.27	0.04	(0.01,0.12)	<0.0001
Raw milk	206	3	0.01	368	6	0.02	0.89	(0.22,3.6)	0.87
Raw cider	200	4	0.02	730	45	0.06	0.31	(0.11, 0.87)	0.02
Foreign travel	222	16	0.07	735	11	0.01	5.11	(2.34,11.19)	<0.0001
Petting zoo	215	26	0.12	385	16	0.04	3.17	(1.66,6.06)	0.0002

Table 2b. Prevalence and odds for exposure in preceding 7 days between cases and controls in females

	Cases			Controls					
Variables	N	Yes	%	N	Yes	%	OR	95% C.I.	p-value
Ground beef	304	198	0.65	1044	574	0.55	1.53	(1.17,1.99)	0.001
Pink burger patties	201	65	0.32	372	119	0.32	1.02	(0.70, 1.47)	0.93
Poultry	271	247	0.91	540	444	0.82	2.23	(1.39,3.57)	0.0007
Salami	280	78	0.28	512	121	0.24	1.25	(0.9,1.74)	0.19
Jerky	283	8	0.03	512	16	0.03	0.9	(0.38,2.13)	0.06
Venison	286	2	0.007	539	12	0.02	0.31	(0.07,1.39)	0.11
Lettuce	268	183	0.68	515	455	0.88	0.28	(0.2,0.41)	<0.0001
Spinach	279	49	0.18	515	126	0.24	0.66	(0.46,0.95)	0.03
Sprouts	280	6	0.02	583	163	0.28	0.06	(0.02,0.13)	<0.0001
Raw milk	285	5	0.02	548	14	0.03	0.68	(0.24,1.91)	0.46
Raw cider	276	6	0.02	1055	37	0.04	0.61	(0.26,1.46)	0.26
Foreign travel	299	21	0.07	1066	10	0.01	7.98	(3.71,17.13)	<0.0001
Petting zoo	288	47	0.16	546	14	0.03	7.41	(4,12.72)	<0.0001

Table 3a. Prevalence and odds for exposure in preceding 7 days between cases and controls, 0-5 year olds

	Cases			Controls					
Variables	N	Yes	%	N	Yes	%	OR	95% C.I.	p-value
Ground beef	96	55	0.57	84	45	0.54	1.16	(0.64,2.1)	0.62
Pink burger patties	63	16	0.25	29	8	0.28	0.89	(0.33,2.41)	0.82
Poultry	93	81	0.87	34	31	0.91	0.65	(0.17,2.48)	0.53
Salami	66	25	0.38	40	10	0.25	1.83	0.77,4.37)	0.17
Jerky	93	1	0.01	40	3	0.08	0.13	(0.01,1.33)	0.05
Venison	93	0	0	35	0	0	undefined		--
Lettuce	84	36	0.43	42	18	0.43	1	(0.47,2.11)	1
Spinach	89	5	0.06	42	6	0.14	0.36	(0.1,1.25)	0.1
Sprouts	90	0	0	35	7	0.2	undefined		<0.0001
Raw milk	92	3	0.03	40	1	0.03	1.31	(0.13,13.04)	0.81
Raw cider	85	1	0.01	85	2	0.02	0.49	(0.04,5.55)	0.56
Foreign travel	96	5	0.05	85	0	0	undefined		0.03
Petting zoo	91	27	0.3	39	2	0.05	7.8	(1.75,34.71)	0.002

Table 3b. Prevalence and odds for exposure in preceding 7 days between cases and controls, 6-17 year olds

	Cases			Controls					
Variables	N	Yes	%	N	Yes	%	OR	95% C.I.	p-value
Ground beef	213	154	0.72	171	92	0.54	2.24	(1.47,3.43)	0.0001
Pink burger patties	153	53	0.35	59	12	0.2	2.07	(1.01,4.25)	0.04
Poultry	185	170	0.92	82	75	0.91	1.06	(0.41,2.7)	0.91
Salami	146	44	0.3	87	35	0.4	0.64	(0.37,1.12)	0.12
Jerky	198	8	0.04	87	8	0.09	0.42	(0.15,1.15)	0.08
Venison	203	0	0	83	1	0.01	undefined		0.12
Lettuce	191	107	0.56	85	59	0.69	0.56	(0.33,0.97)	0.04
Spinach	193	14	0.07	87	17	0.2	0.32	(0.15,0.69)	0.002
Sprouts	195	1	0.005	97	20	0.21	0.02	(0.003,0.15)	<0.0001
Raw milk	198	3	0.02	87	1	0.01	1.32	(0.14,12.9)	0.81
Raw cider	195	5	0.03	173	8	0.05	0.54	(0.17,1.69)	0.29
Foreign travel	209	18	0.09	174	2	0.01	8.1	(1.85,35.44)	0.001
Petting zoo	204	27	0.13	91	3	0.03	4.47	(1.32,15.15)	0.009

Table 3c. Prevalence and odds for exposure in preceding 7 days between cases and controls, ≥18 year olds

	Cases			Controls					
Variables	N	Yes	%	N	Yes	%	OR	95% C.I.	p-value
Ground beef	229	155	0.68	1513	812	0.54	1.8	(1.35,2.43)	<0.0001
Pink burger patties	141	51	0.36	511	159	0.31	1.25	(0.84,1.86)	0.26
Poultry	198	175	0.88	780	633	0.81	1.77	(1.1,2.83)	0.02
Salami	130	68	0.52	741	199	0.27	2.99	(2.04,4.37)	<0.0001
Jerky	204	8	0.04	741	31	0.04	0.93	(0.42,2.07)	0.87
Venison	205	3	0.01	780	22	0.03	0.51	(0.15,1.73)	0.27
Lettuce	196	153	0.78	751	684	0.91	0.35	(0.23,0.53)	<0.0001
Spinach	200	47	0.24	751	175	0.23	1.01	(0.7,1.46)	0.95
Sprouts	202	8	0.04	827	239	0.29	0.1	(0.05,0.21)	<0.0001
Raw milk	201	2	0.01	789	18	0.02	0.43	(0.1,1.87)	0.25
Raw cider	196	4	0.02	1527	72	0.05	0.42	(0.15,1.17)	0.09
Foreign travel	216	14	0.06	1542	19	0.01	5.56	(2.74,11.25)	<0.0001
Petting zoo	208	19	0.09	801	25	0.03	3.12	(1.68,5.79)	0.0002

Table 4a. Prevalence and odds for exposure in preceding 7 days between cases and controls, Summer (June-September)

	Cases			Controls					
Variables	N	Yes	%	N	Yes	%	OR	95% C.I.	p-value
Ground beef	304	213	0.7	557	304	0.55	1.95	(1.45,2.62)	<0.0001
Pink burger patties	206	78	0.39	189	53	0.28	1.56	(1.02,2.39)	0.04
Poultry	276	248	0.9	256	215	0.84	1.69	(1.01,2.82)	0.04
Salami	283	75	0.27	298	73	0.24	1.11	(0.77,1.61)	0.58
Jerky	286	11	0.04	298	18	0.06	0.62	(0.29,1.34)	0.22
Venison	287	2	0.007	257	5	0.02	0.35	(0.07,1.83)	0.2
Lettuce	270	166	0.61	302	259	0.86	0.27	(0.18,0.4)	<0.0001
Spinach	278	31	0.11	302	67	0.22	0.44	(0.28,0.7)	0.0003
Sprouts	283	7	0.02	292	93	0.32	0.05	(0.02,0.12)	<0.0001
Raw milk	284	7	0.02	262	9	0.03	0.71	(0.26,1.94)	0.5
Raw cider	280	5	0.02	560	27	0.05	0.36	(0.14,0.94)	0.03
Foreign travel	300	17	0.06	566	7	0.01	4.8	(1.97,11.7)	0.0002
Petting zoo	291	46	0.16	291	18	0.06	2.85	(1.61,5.04)	0.0002

Table 4b. Prevalence and odds for exposure in preceding 7 days between cases and controls, Winter (December-February)

	Cases			Controls					
Variables	N	Yes	%	N	Yes	%	OR	95% C.I.	p-value
Ground beef	186	115	0.62	452	244	0.54	1.38	(0.97,1.96)	0.07
Pink burger patties	34	9	0.26	142	40	0.28	0.92	(0.39,2.14)	0.84
Poultry	42	40	0.95	241	194	0.8	4.85	(1.13,20.77)	0.02
Salami	41	12	0.29	209	63	0.3	0.96	(0.46,2)	0.91
Jerky	41	1	0.02	209	8	0.04	0.63	(0.08,5.16)	0.66
Venison	42	0	0	240	8	0.03	undefined		0.23
Lettuce	43	26	0.6	213	176	0.83	0.32	(0.16,0.65)	0.001
Spinach	42	4	0.1	212	57	0.27	0.29	(0.1,0.84)	0.02
Sprouts	42	0	0	249	67	0.27	undefined		0.0001
Raw milk	43	0	0	247	4	0.02	undefined		0.4
Raw cider	39	0	0	456	16	0.04	undefined		0.23
Foreign travel	46	4	0.09	777	2	0.002	36.9	(6.57,207.25)	<0.0001
Petting zoo	44	3	0.07	252	3	0.01	6.07	(1.19,31.12)	0.01

Table 4c. Prevalence and odds for exposure in preceding 7 days between cases and controls, Spring/Fall (March-May, October-November)

	Cases			Controls					
Variables	N	Yes	%	N	Yes	%	OR	95% C.I.	p-value
Ground beef	48	36	0.75	759	401	0.53	2.68	(1.37,5.23)	0.003
Pink burger patties	114	33	0.29	268	86	0.32	0.86	(0.53,1.39)	0.54
Poultry	158	138	0.87	399	330	0.83	1.44	(0.84,2.47)	0.18
Salami	166	50	0.3	361	108	0.3	1	(0.68,1.51)	0.96
Jerky	168	5	0.03	361	16	0.04	0.66	(0.24,1.84)	0.42
Venison	168	1	0.006	401	10	0.02	0.23	(0.03,1.84)	0.13
Lettuce	158	104	0.66	363	326	0.9	0.22	(0.14,0.35)	<0.0001
Spinach	162	31	0.19	366	74	0.2	0.93	(0.59,1.49)	0.77
Sprouts	162	2	0.01	418	106	0.25	0.04	(0.009,0.15)	<0.0001
Raw milk	164	1	0.006	407	7	0.02	0.35	(0.04,2.87)	0.31
Raw cider	157	5	0.03	769	39	0.05	0.62	(0.24,1.59)	0.31
Foreign travel	175	16	0.09	775	12	0.02	6.4	(2.97,13.79)	<0.0001
Petting zoo	168	24	0.14	389	9	0.02	7.04	(3.19,15.5)	<0.0001

Table 5. Mantel-Haenzel adjusted, age-stratified odd ratios for 'petting zoo' and 'foreign travel' risk factors

	Cases			Controls					
Ages	N	Yes	%	N	Yes	%	OR	95% C.I.	p-value
Petting zoo									
0 to 5	91	27	0.3	39	2	0.05	7.8	(1.75, 34.71)	0.002
6 to 17	204	27	0.13	91	3	0.03	4.47	(1.32,15.15)	0.009
≥18	208	19	0.09	801	25	0.03	3.12	(1.68,5.79)	0.0002
All groups (adjusted)	503	73	0.15	931	30	0.03	4.06	(2.42,6.83)	<0.0001
Foreign travel									
0 to 5	96	5	0.05	85	0	0	undefined		0.03
6 to 17	209	18	0.09	174	2	0.01	8.1	(1.85,35.44)	0.001
≥18	216	14	0.06	1542	19	0.01	5.56	(2.74,11.25)	<0.0001
All groups (adjusted)	521	37	0.07	1801	21	0.01	6.66	(3.36,13.19)	<0.0001

Table 6. Prevalence and odds for exposure to poultry in preceding 7 days between cases and controls, by ground beef consumption (≥18 years)

	Cases			Controls					
Variables	N	Yes	%	N	Yes	%	OR	95% C.I.	p-value
Poultry (no ground beef)	45	37	0.82	353	293	0.83	0.95	(0.42, 2.14)	0.9
Poultry (ground beef)	142	128	0.9	406	329	0.81	2.14	(1.17,3.92)	0.01

Table 7. Prevalence and odds for exposure to lettuce in preceding 7 days between cases and controls, by ground beef consumption

		Cases			Controls					
Ages	Variables	N	Yes	%	N	Yes	%	OR	95% C.I.	p-value
0 to 5	Lettuce (no ground beef)	27	8	0.27	14	13	0.93	0.03	(0.004, 0.29)	0.0001
	Lettuce (ground beef)	48	26	0.54	18	16	0.89	0.15	(0.03,0.71)	0.009
6 to 17	Lettuce (no ground beef)	37	23	0.62	38	30	0.79	0.45	(0.16,1.22)	0.11
	Lettuce (ground beef)	142	76	0.54	47	41	0.87	0.17	(0.07, 0.42)	<0.0001
≥18	Lettuce (no ground beef)	46	35	0.76	356	311	0.87	0.46	(0.22,0.97)	0.04
	Lettuce (ground beef)	140	111	0.79	386	336	0.87	0.57	(0.34, 0.94)	0.03

Table 8. Prevalence and odds for exposure to spinach in preceding 7 days between cases and controls, by ground beef consumption

		Cases			Controls					
Ages	Variables	N	Yes	%	N	Yes	%	OR	95% C.I.	p-value
0 to 5	Spinach (no ground beef)	29	1	0.03	18	3	0.17	0.18	(0.02,1.87)	0.11
	Spinach (ground beef)	50	3	0.06	22	4	0.18	0.29	(0.06,1.41)	0.11
6 to 17	Spinach (no ground beef)	37	5	0.14	28	5	0.18	0.72	(0.19,2.77)	0.63
	Spinach (ground beef)	146	9	0.06	57	11	0.19	0.32	(0.13,0.81)	0.01
≥18	Spinach (no ground beef)	46	10	0.22	360	79	0.22	0.99	(0.47, 2.08)	0.97
	Spinach (ground beef)	143	32	0.22	383	92	0.24	0.91	(0.58,1.44)	0.69

VIII. Appendix I

I. Dietary, recreational and drinking water, travel and occupational exposure questions from the *E. coli* O157 and Other Shiga toxin-producing *E.coli* questionnaire used for cases

Exposure variable (7 days prior to onset of STEC infection)
Poultry and meats • Ground beef • Jerky • Other meat • Pink burger • Poultry • Salami • Venison • Burgers at home **Fresh vegetables** • Bagged lettuce • Lettuce • Spinach • Sprouts **Unpasteurized dairy and juices** • Unpasteurized cider • Unpasteurized milk **Recreational activities** • Swim in pool • Swim in lake • Visited petting zoo **Travel** • Any traveling outside of the US **Occupational/residential** • Live in farm • Employment involves handling food • Attends a childcare center

II. Dietary, recreational and drinking water and travel exposure questions from the FoodNet Population Survey Adolescent, Adult and Pediatric Questionnaire 2006-2007 used for controls

Exposure variable (7 days prior to survey interview)
Poultry and meats • Chicken at home • Whole-chicken meal at home • Pre-cut chicken parts meal at home • Ground chicken • Ground turkey • Other turkey • Pre-frozen beef patties eaten at home • Fresh beef patties eaten at home • Pink patties eaten at home (pre-frozen/fresh) • Ground beef • Any kind of game (i.e. venison, pheasant, etc.) • Pepperoni/salami • Store-bought jerky **Fresh vegetables** • Alfalfa sprouts • Bean sprouts • Other sprouts • Any salad with lettuce/greens • Salad mix • Mesclun lettuce • Iceberg lettuce • Romaine lettuce • Other leaf lettuce • Lettuce in burgers/sandwiches • Fresh spinach **Unpasteurized dairy and juices** • Unpasteurized cider • Unpasteurized milk **Recreational activities** • Visited petting zoo **Travel** • Any traveling outside of the US