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DESCRIPTIVE EPIDEMIOLOGIC AND MICROBIOLOGIC STUDY OF SALMONELLA TENNESSEE ISOLATES ASSOCIATED WITH PEANUT BUTTER- NATIONAL OUTBREAK OF 2006-2007

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DESCRIPTIVE EPIDEMIOLOGIC AND MICROBIOLOGIC STUDY OF *SALMONELLA* TENNESSEE ISOLATES ASSOCIATED WITH PEANUT BUTTER- NATIONAL OUTBREAK OF 2006-2007

By

Chau Hong Nguyen

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ABSTRACT

DESCRIPTIVE EPIDEMIOLOGIC AND MICROBIOLOGIC STUDY OF SALMONELLA TENNESSEE ISOLATES ASSOCIATED WITH PEANUT BUTTER-NATIONAL OUTBREAK OF 2006-2007

By

Chau Hong Nguyen

The number of outbreaks of foodborne bacterial diseases increases each year due to newly emerging strains of pathogens or food vehicles that have not been previously identified. The multi-state outbreak of *Salmonella* serotype Tennessee infections associated with peanut butter, 2006-2007 was the first outbreak in the United States associated with this food vehicle. Results of our investigation revealed that *S.* Tennessee strains associated with the peanut butter outbreak are more likely to be highly invasive than strains from non-outbreak sources, odds ratio (OR) = 4.025 (p-value=0.0088, 95% confidence interval (CI): (1.419, 11.418). Descriptive epidemiologic study revealed that *S.* Tennessee strains were more likely to be isolated from females than from males.

Results of the epidemiologic and virulence characterization suggested that peanut butter could have served as a conducive medium for *S.* Tennessee to express certain sets of virulence genes leading to the observed high level of invasiveness. The occurrence of this outbreak highlights the importance of hygienic practices in peanut butter manufacturing plants for the prevention of such mass contamination.

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LIST OF ABBREVIATIONS

OR	Odds Ratio
CI	Confidence Interval
CDC	Centers for Disease Control and Prevention
WHO	World Health Organization
DT104	Definitive Type 104
PFGE	Pulse Field Gel Electrophoresis
FDA	Food and Drug Administration
PCA	Peanut Corporation of America
Caco-2	adenoCArcinoma of the Colon
ATCC	American Type Culture Collection
DMEM	Dulbecco's Modified Eagles Medium
FBS	Fetal Bovine Serum
TSA	Tryptic Soy Agar
BHI	Brain Heart Infusion
PBS	Phosphate Buffer Saline
IGM	Intracellular Growth Medium
MLST	Multi-locus Sequence Typing
ST	Sequence Type
USDA	United States Department of Agriculture

BACKGROUND

Foodborne infections:

Foodborne bacterial infections are among the most common infections encountered by millions of people all around the world. These infections occur due to consumption of food or water contaminated with bacterial pathogens or their toxins, introduced into food products due to failure of effective implementation of food safety principles during pre and post harvesting stages of food (1). These stages include food production, transportation, processing, distribution, preparation, and consumption (2). In 2006, more than 3,300 foodborne illnesses were reported to the Centers for Disease Control and Prevention's (CDC) Foodborne Disease Outbreak Reporting System (3). Foodborne infections have emerged to become a worldwide problem (2). Common bacterial pathogens associated with foodborne infections are *Salmonella*, *Campylobacter*, *Listeria*, and *Escherichia coli* (4). An estimate of 76 million illnesses each year in the United States are due to foodborne infections, of which 325,000 lead to hospitalization and 5,000 lead to death (5, 6). Surprisingly, of the 76 million foodborne illnesses, only 14 million are caused by known agents, whereas the causative pathogens of the remaining 62 million are unknown (6). Complete statistics of the frequency of foodborne illnesses for calculation of incidence along with global burden and cost of the disease have continued to be a challenge for investigators due to underreporting of the disease, or newly emerging pathogens or agents that has not been identified previously (5, 6, 7). Continuous efforts are required by public health investigators for improvement of food safety and development of effective prevention strategies to decrease the occurrence of foodborne infections (5, 6).

SALMONELLA

Salmonella is a gram-negative, rod-shaped, facultative anaerobe organism with motile peritrichous flagella belonging to the family Enterobacteriaceae (8, 9, 10, 11, 12). Its facultative anaerobe characteristic permits the bacteria to a wide range of growth and activity in nutrient-poor, acidic, low water activity environments, for long periods (12). *Salmonella* can exist and survive almost anywhere from contaminated soil lasting over 200 days, to 10 months in dust, and over 4 years in dried whole eggs (13). Interaction with food at temperature from 7 to 46° C can cause multiplication of the organism (13).

Salmonella was named after D.E Salmon, an American bacteriologist and veterinarian in 1886 when the first isolation of "hog cholera bacillus" was reported from diarrheic swine (12, 13, 14, 15). S. Enteritidis was isolated three years later from the meat and spleen of a man who died after consuming raw meat from an ill cow, reported from the first nontyphoidal salmonellosis foodborne outbreak (13). *S.* Typhimurium was first isolated and reported in 1889 by Loeffler, from an ill laboratory mouse colony (13).

Taxonomy and nomenclature:

The taxonomy and nomenclature for *Salmonella* has been in consistent debate among international countries over the years (12, 13, 14). In accordance with the World Health Organization (WHO), it is now accepted that there is only one single species of *Salmonella* called S. enterica with seven subspecies: I, II, IIIa, IIIb, IV, V, VI (12, 14, 16). The Kauffmann-White scheme is a detailed antigenic formulae and grouping of each *Salmonella* serotype adopted for use in 1934 (14). Different strains and isolates of

Salmonella species are classified and assigned specific names based on the Kauffmann-White scheme of serological identification (11, 12).

According to F. Kauffmann, "the species [genus *Salmonella*] is a group of related sero-fermentative phage-types or a group of related sero-, bio-, phago- (or lyso-) types" (17). Different antigens are expressed on the surface of the bacterial cells (18). Each *Salmonella* serotype possess a unique antigenic formula derived from the combination of antigens expressed (18). Somatic or outer membrane antigens are known as O antigens, H is classified as the flagella antigens, and very few *Salmonella* bacterial cells produce the capsular antigens, Vi (18). The name for each *Salmonella* serotype is related to the disease caused to the host or after the place in which the organism was first isolated (14). This latter approach is more commonly used for naming new *Salmonella* types (14).

Pathogenesis:

The pathogenic spectrum for *Salmonella* infections consist of various steps displayed in figure 1. Transmission of the bacteria to a susceptible host is the first step in the disease process (16). Upon entry into the host via ingestion, the bacteria must survive the stomach acidity in order to initiate an infection (12). Initiation of an infection occurs when the bacteria attaches to the small intestine and penetrates the mucosa layer of the intestine for entry into the midlayer, and are then engulfed into the epithelial cells achieved with the help of adhesins (12). The organism will penetrate the epithelial cells into the small bowel and colon causing an inflammatory response leading to inflammation and destruction of tissues (12). Furthermore, different serovars can

penetrate further into the deeper layers of the mucosa of the intestinal wall (12). Inflammatory reactions elicited in the small intestine results in fever and diarrheal symptoms, observed in most infected patients (12).

Clinical manifestations:

Salmonella infections can cause a wide range of diseases in humans: enteric fever, gastroenteritis, and bacteremia (11, 19). Gastroenteritis is the most common disease among the three (11). Symptoms associated with *Salmonella* infections consist of fever, diarrhea, abdominal pains, nausea, and occasionally vomiting that develop in 12 to 72 hours after infection and can last from 4 to 7 days or prolonged (20, 21). In most individuals, the disease is not life threatening and patients will recover without requiring treatment (20, 21). However, in some patients, especially the young, elderly, and immunocompromised individuals, the disease can be severe requiring hospitalization or treatment with antimicrobials such as ampicillin, trimethoprim-sulfamethoxazole, or ciprofloxacin (20, 21). Additionally, bacteremia and focal infections are more likely to develop in immunocompromised individuals (19). Complete recovery of bowel habits is usually observed in individuals who develop diarrhea (21). In a small number of diseased individuals, a condition known as Reiter's syndrome can develop causing irritation in the eyes, painful urination, and pains in the joints, lasting for months or years leading to development of chronic arthritis (20).

SALMONELLOSIS

Salmonellosis is defined as "an infection with any serotype of the *Salmonella* bacteria" (20).

Among the widely distributed foodborne diseases, salmonellosis is one of the most common, occurring worldwide (21, 22). Small *Salmonella* outbreaks among the general population are usually contained. Large outbreaks have been reported in hospitals, schools, restaurants, and institutions (22). Over 40,000 cases of salmonellosis are reported to the CDC each year in the United States (22). However, this represents the tip of the iceberg since based on systemic investigations by the CDC-FoodNet, *Salmonella* serotypes infect approximately 1.4 million people annually in the United States, resulting in greater than 100,000 physician office visits, over 16,000 hospitalization, and 2.3 billion in medical care and productivity costs (23).

Causes of salmonellosis:

Most of *Salmonella* serotypes can cause illness in humans and animals. However about 20 serotypes are more common reported in salmonellosis than other serotypes. Of these serotypes, *S.* Enteritidis and *S.* Typhimurium are the most frequent (22). *S.* Enteritidis has become the most common cause of food poisoning in the United States within the last twenty years (15). According to the WHO Global Salm-Surveillence, from 2000-2002, *S.* Enteritidis was the most common serotype among human isolates, accounting for 65% of all isolates; consequently, making it the dominant serotype of human isolates not only in the United States but also in other industrialized nations (13, 24).

S. Typhimurium, including the Definitive Type 104 (DT104) strains is the second most common strain and has been found to be resistant to several antimicrobials including ampicilin, choramphenicol, streptomycin, sulfamethoxazole, and tetracycline (25). These classes of antimicrobials are commonly used in treatment of human and animal infections. (22). Multi-drug resistant *S.* Typhimurium, (DT104), constituted 7% of human *Salmonella* infections in the United States in 1998 (26). Nontyphoidal salmonellosis became a public health event in the United States after World War II (13). In 1942, when salmonellosis was fist nationally reported, a total of 502 clinical cases were observed (13). The number of reported cases of salmonellosis in the United States from 1976-1999 are displayed in figure 2 (27). The highest number of reported cases observed was in 1985 with approximately more than 65,000 cases. Reported cases of salmonellosis have remained relatively stable within the last 10 years (27). The reported incidence of salmonellosis in the United States from 1997-2007 are shown in figure 3. The incidence rates observed within the 10-year period has remained within a range of 14.39 to 16.03 per 100,000

Transmission of *Salmonella* serotypes:

Salmonella infections in human is acquired via consumption of contaminated water and food of animal origin or produce contaminated with animal products (7, 21). Acquisition of *Salmonella* can also come from handling pets (22, 28, 29) Exposure to *Salmonella* in the household can home when food products, particularly of animal origin, are prepared poorly and inadequately cooked before consumption (22, 30). Moreover, food can also be contaminated by people who are carriers and does not follow appropriate hygienic

methods when preparing meals, followed by inadequate or no cooking, allowing the survival of the contaminants and their multiplication to a minimum infectious dose (12).

Primarily, *Salmonella* are reservoired in animals and the environment. *Salmonella* serotypes have been isolated from most animal species particularly, reptiles. Additionally, *Salmonella* are also widespread in the environment, in areas such as water and soil, with long survival periods without multiplication (16). Animals are infected with *Salmonella* through consumption of contaminated feed and water (12). The estimated required dosage of the bacteria for disease initiation is 10^5 to 10^{10} bacteria, however, as low as 10 organisms can initiate an infection (13, 16). Moreover, other factors are also considered, such as the type of strain, the amount of contaminated food consumed, and the physiological state of the host at time of infection (16).

Prevention of salmonellosis:

Various measures can be taken to prevent from *Salmonella* infections, such as avoiding consumption of raw or undercooked food products and unwashed raw produce (22). Other measures include careful hand washing after handling pets, especially reptiles, and washing all food surfaces and utensils used for food preparation thoroughly (22). All food, especially meat, should be cooked thoroughly to the required cooking temperature before consumption because food contaminated with *Salmonella* will look and appear normal, therefore making it difficult to detect by the naked human eye (22).

SALMONELLA SEROTYPE TENNESSEE

Outbreaks of foodborne illness are commonly caused by consumption of food contaminated with *Salmonella* (14). *Salmonella* outbreaks were associated with various groups of food vehicles (listed in Table 1). There are over 2,500 identified *Salmonella* serotypes of which 20 are commonly associated with human salmonellosis (21, 31). *S.* Tennessee is not a common serotype, thus infections are rare, and the average numbers of reported cases each year during 1994-2004 were approximately 52 cases (31). The average reported cases for *S.* Tennessee infections represent 0.01% of all reported *Salmonella* serotypes (31). There has only been one earlier outbreak of *S.* Tennessee infections associated with powered milk products and infant formula in the United States and Canada, reported to CDC in 1993 (32, 33).

In November 2006, a substantial increase in the incidence of *S.* Tennessee infections was reported from most of the United States. Subsequent investigation by the states health authorities, CDC, and the FDA demonstrated that peanut butter consumption was incriminated in most cases, underscoring the emergence of peanut butter as a new food vehicle for human salmonellosis in the United States (31, 34, 35).

Salmonella serotype Tennessee outbreak associated with peanut butter, United States, 2006-2007: (3, 31, 34)

On national bases, average numbers of *S.* Tennessee isolates were generally from 1-5 isolates per month, whereas, in October of 2006, the numbers of reported isolates increased to 30 isolates per month. A case-control study was then conducted during

February 5- 13, 2007 leading to identification of consumption of peanut butter with Peter Pan or Great Value brand as the cause of the illness. Both of these brands were produced at the same manufacturing plant, Sylvester, Georgia, and *S.* Tennessee strains were isolated from several unopened and opened jars of the two brands. This outbreak leads to the emerging and identification of peanut butter as a new food source for salmonellosis in the United States.

Based on the *Salmonella* Annual Summary of 2006, the numbers of *S.* Tennessee isolates reported more than doubled from 2005 to 2006 due to this first reported outbreak of *S.* Tennessee resulting from contaminated peanut butter exposure in the United States. The outbreak caused more than 800 cases in 47 states, accounting for the observed increase in reported cases within the year. PulseNet serotyping of the number of isolates reported from the outbreak strain identified 3 closely related pulsed-field gel electrophoresis patterns (PFGE): JNXX01.0001, .0011, and .0026.

CDC officials defined a case for this outbreak as "infection with *Salmonella* Tennessee with a PFGE pattern matching one of the three outbreak patterns (ending in .0001, .0011, or .0026) in a person residing in the United States with symptoms onset on or after August 1, 2006". The case findings for *S.* Tennessee infections associated with peanut butter as reported by CDC are listed in table 2. Of all patients, the median age was 52 years, ranging from 2 months to 95 years of age. Seventy-three percent of the patients were female compared to 27% for males. Sources of isolates came from stools (61%), urine (35%), and other specimens (4%). Additionally many reported symptoms of

diarrhea (72%), abdominal cramps (65%), fever (43%), and dysuria (45%). The high percentage of cases reported for dysuria suggests that *S.* Tennessee is more likely to infect the urinary tract than other serotypes.

A case-control study conducted by state and local health departments to identify the food items associated with illness obtained 64 cases and 124 controls (Table 3). The median age for case patients was 53 years, and 58 years for controls. It was observed that cases were more likely than controls to have consumed peanut butter (81% versus 65%) (Table 3). Additionally, patients were also more likely than controls to have eaten either Peter Pan or Great Value peanut butter (67% versus 13%) (Table 3). Epidemiologic data provided to the Food and Drug Administration (FDA) officials suggested Peter Pan peanut butter as the possible source of the outbreak. On February 14, 2007, a recall was implemented for both brands, resulting in a decline of reported cases .

***Salmonella* serotype Typhimurium outbreak associated with peanut butter, United States, 2008-2009: (35, 36)**

A second national outbreak associated with peanut butter contaminated with *Salmonella* Typhimurium in which larger numbers of children were infected was reported between 2008-2009. Infected individuals ranged from <1 to 98 years of age, with a median age of 16 years. Twenty-one percent of illnesses occurred in individuals <5 years of age. Forty-eight percent of patients were females and 22% were hospitalized.

Interviews conducted with infected patients revealed that the outbreak occurred within 3 large institutions (2 care facilities and 1 elementary school) where the patients ate their meals. Further investigation and review of food menus revealed a common food source eaten by infected patients: King Nut creamy peanut butter produced by Peanut Corporation of America (PCA). Interestingly, during this outbreak investigation, CDC's PulseNet identified and confirmed *Salmonella* strains of other serotypes other than Typhimurium, found in food and environmental samples (Figure 4). It was reported that an *S.* Tennessee isolate detected during this outbreak was noted to have a PFGE pattern indistinguishable from the 2006-2007 *S.* Tennessee outbreak strains, obtained from unopened and opened jars of King Nut brand peanut butter from Georgia and Minnesota, leading to a possible association between the two outbreaks. Furthermore, the two implicated plants are located approximately 70 km from one another. However, *S.* Tennessee strains from this outbreak were not associated with an increase in illness in humans.

PFGE profiles of peanut butter derived isolates: (31, 35)

Peanut butter derived isolates have unique PFGE profiles. *S.* Tennessee isolates from the 2006-2007 outbreak displays three closely related PFGE patterns: JNXX01.0010, JNXX01.0011, and JNXX01.0026. *S.* Typhimurium on the other hand, displayed 2 clusters of unusual PFGE patterns. The first cluster consisted of 13 *S.* Typhimurium isolates with PFGE pattern JPXX01.1818. The second cluster consisted of 41 *S.* Typhimurium isolate with PFGE patterns JPXX01.0459/JPXX01.1825. Isolates from the two clusters were noted to be similar in patterns and testing of the isolates confirmed that

the two clusters displayed the same pattern: JPXA26.0462. A different sub-typing method, multilocus variable-number tandem-repeat analysis, showed that the two isolates were indistinguishable and were epidemiologically similar. As a result, the two clusters are grouped together as a single outbreak strain.

***Salmonella* contamination of peanuts:**

Contamination of peanuts with *Salmonella* can occur during growth, harvest, or storage of peanuts (31). Peanuts can easily be contaminated due to its low water activity and high fat content characteristics in which *Salmonella* favors and can survive indefinitely (31, 35). Although peanuts are initially roasted at 350 ^{o} F to sufficiently kill existing *Salmonella*, however, these organisms can be reintroduced after heat treatment in the post-processing steps from salmonellae brought into the plant on raw peanuts, humans from outside environment, or animals around and in the production environment (35).

The *S.* Tennessee outbreak in peanut butter was the first outbreak in the United States implicated for this broadly distributed food item (31). The *S.* Tennessee and *S.* Typhimurium outbreaks outline peanut butter as a potential cause of widespread illness (35). Additionally, these two outbreaks have planted upon manufacturing plants an important message: even when a heat-treatment step is used, processed food can become contaminated again. Food-processing plants have underscored this prevention measure, leading to contamination of processed food as a result (48).

Recall of peanut butter and peanut containing products: (37, 38)

Peanut butter and peanut butter paste are widely distributed to various distributors and are common ingredients in products such as cookies, crackers, cereal, candy, ice cream, pet treats, and food toppings, consumed on a daily basis. The *S.* Tennessee and *S.* Typhimurium outbreaks associated with peanut butter in the United States has lead to the recall of various food items. A peanut butter and other peanut containing products recall list generated by the FDA in January of 2009 entails more than 2,100 products in 17 categories of which are recalled by more than 200 companies (Table 4). The recall list includes not only human food but also pet food related to peanut products. Over $30,000 worth of peanuts and peanut products containing PCA peanuts was seized by United States Marshall at Westco/Westcott due to possible *Salmonella* contamination. The recall of peanut butter and peanut containing food products have resulted in the PCA filing for bankruptcy.

As a result of the *S.* Tennessee and *S.* Typhimurium peanut butter outbreaks, retailers were advised by the FDA to stop selling recalled products. Institutions and food service establishments were asked to check their food products to ensure that they were not serving any food listed on the recall list. Customers were advised to consult their health care providers if they have been ill from eating peanut products. Customers were also informed to throw away any peanut products in the home that are listed on the recall list or will be recalled, and avoid consumption of any peanut food products that they think are potentially contaminated. Manufactures were asked to inform customers through the FDA website whether or not their products contain peanuts from the PCA.

Societal impacts of the peanut butter-associated outbreaks: (39)

Peanut butter and peanut containing food products became an important concern and the outbreaks were the beginning of a food safety crisis. Due to the recall issued by the FDA for manufactures and retailers to remove potentially contaminated products off their shelves, this resulted in a dramatic decline in the sales of peanut butter and peanut containing products. Stores across the country experienced approximately 25.9% decrease in the number of peanut sales. As a result of the *Salmonella* outbreaks associated with peanut butter, peanut butter has left a negative impact on consumers of this product. Extra measures should be taken by manufactures and retailers to educate consumers and diminish any negative impact.

VIRULENCE TYPES AND PHYLOGENETIC ANALYSIS OF *SALMONELLA* TENNESSEE ISOLATES

As stated previously, the primary entry mode for *Salmonella* into its host is ingestion of the bacteria. Once ingested, the bacteria can reach underlying sites by passing through the intestinal lumen (40). It is noteworthy that "all *Salmonella* serotypes share the ability to invade the host (41)." However, the ability of each *Salmonella* serotypes to attach and induce its own uptake into cells of the intestinal lumen depends on various factors such as its growth state (40) and the outer surface proteins expressed (42). The specific characteristic that each *Salmonella* serotype possess contribute and influence its invasiveness (42). Additionally, the ability of each serotype to establish an infection with its host relies on its ability to attach, colonize, and invade the host's epithelial cells upon initial contact with the epithelial cells (42). Moreover, although *Salmonella* isolates are closely related, each strain differs in the degree of attachment and invasiveness to intestinal cell lines (43).

The attachment and invasiveness of *S.* Tennessee isolates associated with the national peanut butter outbreak were studied using tissue culture assays. Tissue culture is a technique developed in the 1940s for assessment of animal cells as in vitro systems (44). Used in many areas of science, this technique enables scientists and researchers to induce and grow cells outside of the host (44). Development of tissue culture techniques allow for assessment of a specific cell line growth, differentiation, and its specific role and function as if it was in the host cell (45). Furthermore, tissue culture systems have been established as models for use to study the attachment, invasion, and virulence

mechanisms of different *Salmonella* serotypes entry into mammalian cells (40, 42). Different mammalian cells such as Henle cells, Caco-2 cells, and Hep-2 cells are used in tissue culture systems for studying and understanding the natural processes of *Salmonella* infections (44). Caco-2 cells (adenoCArcinoma of the Colon) are immortalized cell line derived from human epithelial colorectal adenocarcinoma cells (42). When grown as a monolayer to 80% confluency, Caco-2 cells will differentiate and resemble columnar epithelial cells (46, 47, 48), of which can be use in attachment and invasion assay of bacterial cells.

STUDY OBJECTIVES AND METHODS

The emergence of the national outbreak of *Salmonella* serotype Tennessee associated with peanut butter in 2006-2007 has been the focus of this descriptive epidemiologic and microbiologic study of *S.* Tennessee isolates associated with the national peanut butter outbreak.

Objective 1: To describe the epidemiologic attributes of clinical cases:

Salmonellosis is a notifiable disease; therefore, cases are required to be reported to community health departments for maintaining an updated disease record (49, 50). In this study, *S.* Tennessee isolates were collected from several participating state Departments of Health with epidemiological data when available. Sources are listed below:

1. Seventeen isolates from Michigan Department of Community Health,
2. Nineteen isolates from Cornell University and New York Department of Health,
3. Seven isolates from Indiana Department of Health,
4. Thirty nine isolates from Tennessee Department of Health,
5. Fifty isolates from Minnesota Department of Health.
6. Thirty seven *S.* Tennessee isolates were received from University of Pennsylvania, and
7. Twenty three isolates were received from the USDA National Veterinary Service Laboratory (NVSL) Ames. Iowa. These samples included of 22 isolates from animal sources and 1 isolate was from animal feed.

Two references *S.* Tennessee isolates were received from University of Calgary. A total of 194 isolates were procured. We will use SAS systems ver 9.1.3 to perform descriptive epidemiologic study of the association between certain demographic epidemiologic attributes and invasive *S.* Tennessee strains.

Objective 2: Virulence profiling of *Salmonella* Tennessee isolates using attachment and invasion patterns of Caco-2 cells model grown in tissue culture:

We conducted virulence profiling of the *S.* Tennessee isolates using Caco-2 cells model grown in tissue culture to perform attachment and invasion assays. The Caco-2 cells used for this tissue culture project was isolated from a primary colonic tumor of a 72 year old Caucasian male (ATCC HTB-37), and expresses characteristics of enterocytic differentiation upon reaching 80% confluency. Cells were grown in complete growth medium comprised of Dulbecco's Modified Eagles Medium (DMEM) supplemented with 20% (vol/vol) Fetal Bovine Serum (FBS), 1% (vol/vol) nonessential amino acids, and antibiotics to final concentrations of 100 ug/ml penicillin and 100 ug/ml streptomycin. Cells were maintained at 37°C in 5% CO_2 and 95% air in T-75 flasks (Sarstedt, Numbrecht, Germany) containing 10 ml of complete growth media for Caco-2 cell lines. Cells were grown, feed every 2-4 days, and subcultured when reached 80% confluency using trypsin to detach cells from flask wall.

S. Tennessee isolates were identified and labeled in preparation for attachment and invasion assay. Two days before attachment or invasion, selected *S.* Tennessee isolates were grown on Tryptic Soy Agar (TSA) plates. Next day, an isolated colony was picked

and inoculated into 10 ml Brain Heart Infusion (BHI) broth tubes for bacterial growth overnight. Prior to attachment or invasion assay, each BHI tubes were vortexted and each bacterial isolate was plated to determine bacterial counts by dilution.

Attachment assays were performed using isolates grown with and without 1% D-Mannose in the medium. For attachment assays, each individual wells in 24-well plates were seeded with 3.31×10^5 Caco-2 cells and grown as a monolayer on sterile cover slips to 80% confluency. Prior to attachment, wells were washed twice with incomplete DMEM (no FBS or antibiotics) (Gibco), and approximately 5×10^8 bacterial cells grown with and without 1% D-mannose were added to cover slips in each well with 2 ml of complete DMEM containing no antibiotics. Twenty-four well plates were then incubated for 1 hr in a 5% CO_2 atmosphere at 37 °C. After 1 hour, cells in 24-well plates were washed 3 times with sterile Phosphate Buffer Saline (PBS), fixed with methanol for 15 minutes, and stained with a 1:7 dilution Giemsa stain (Sigma) for 1 hour. Each cover slip was then removed from the wells, rinsed with PBS twice and a final rinse with distilled water and hung in coupling jars overnight to dry. Cover slips were mounted using Permount (Fisher) on microscope slides (Fishers) and left to dry overnight for examination by light microscopy the following day.

Invasion assays with Caco-2 cells were performed as described above except incubation period was 3 hours. After 3 hours of incubation, each 24-well plates were washed once with complete DMEM and intracellular growth medium (IGM) consisting of DMEM, 20% FBS, and 1 ml of Gentamicin per 100 ml was added and plates incubated. Plates

and inoculated into 10 ml Brain Heart Infusion (BHI) broth tubes for bacterial growth overnight. Prior to attachment or invasion assay, each BHI tubes were vortexted and each bacterial isolate was plated to determine bacterial counts by dilution.

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were replaced every hour with new IGM for 3 hours. After 3 hours of incubation, plates were performed as described above for attachment. The purpose of addition of Gentamicin in invasion assays is to kill any extra-cellular bacteria without affecting growth of intracellular bacteria (51).

Objective 3: Genotypic characterization of *S.* Tennessee isolates by Multi-Locus Sequence Typing (MLST):

Seven housekeeping genes derived from the *Salmonella* multi-locus sequence typing (MLST) database was used for genotypic characterization of 60 *S.* Tennessee isolates by MLST. These housekeeping genes are listed in table 5 and displayed in figure 5.

MLST is a technique used to characterize and categorize bacteria, providing a standardized approach to data collection (52). The technique of MLST involves PCR amplification of fragments followed by DNA sequencing with either the forward or reverse PCR primer (52). The sequencing step of MLST will identify the variation (polymorphic site) at a locus and assign a specific allele number for the observed polymorphic site of the gene (53, 54). The alleles at the chosen 7 housekeeping gene loci provide an allelic profile of the gene which in turn defines the sequence type (ST) of each isolate (53). It is noted that isolates of the same serotype will display the same ST (54). The sequencing fragments of the 7 housekeeping genes ranges from 430-500 base pairs (53). Housekeeping genes of this size are chosen because it has been demonstrated that DNA fragments of this length can be used in most bacterial pathogens for accurate sequencing and identification of many different alleles within the population (53).

Distinction of genetic variation among isolates allows for characterization of bacterial isolates (55). The procedure of MLST enables the examination of the relationship among isolates by comparing their allelic profiles.

STATISTICAL ANALYSES

Descriptive analysis:

SAS Proc freq procedure was used to compute frequency and percentages for each categorical variable of interest. This procedure was also used to produce cross tabulations tables to test for association of distribution of isolation sites of isolates by age groups and gender of cases.

Univariable analysis:

Each variable was categorized as binary, ordinal, or multinomial variables. Proc logistic procedure was used to perform unadjusted analysis of each individual variable of interest with the outcomes.

One of the main variables of interest is outbreak. *S.* Tennessee strains with PFGE pattern provided from participating states Department of Health ending in JNXX01.0010, .0011, or .0026 were classified as associated with cases having a positive exposure to peanut butter and related to the outbreak. *S.* Tennessee strains having other PFGE patterns were labeled as non-peanut butter associated and was used as the reference group.

Age was analyzed as a categorical variable with 4 groups: <5 years, =5 and <16 years, =16 and <65 years, and =65 years of age. Age group of =16 and <65 years was used as a reference group. Male was used as the reference group for the variable gender.

Another variable of interest was site of isolation. Three groups were formed for this variable: stool, urine, and other. The group other consisted of blood and wound sites. These two sites were merged together to meet the convergence criteria for analysis because the counts observed for blood and wound individually were small.

There are two main outcome variables of interest: attachment and invasiveness. The outcome variable attachment was coded as 0 for negative attachment results and 1 for positive attachment results of bacterial cells with Caco-2 cell models grown in tissue culture. The interaction was assessed under a light microscope.

The second outcome variable of interest is invasiveness. Invasiveness results were assigned to 2 categories based on the percentage of Caco-2 cells containing bacterial micro-colonies as observed under a light microscope. Outcome for invasion was coded as either highly invasive (greater than or equal to 75% of Caco-2 cells containing bacterial micro-colonies as observed under a light microscope) or invasive (between 25 and 75% of Caco-2 cells containing bacterial micro-colonies as observed under a light microscope).

Multivariable analysis:

In addition to the univariable analysis, a multivariable analysis was performed to test for possible interactions. Possible interactions were: age and outbreak, gender and outbreak, and site of isolation and gender. Variables that were tested for confounding was age and gender. Confounding variables were tested by observing the percent change in the

unadjusted and adjusted OR estimates of the variables of interest. If the OR estimates for the tested variable changed by more than 10%, this variable was considered as a confounder.

RESULTS

We have performed attachment and invasion profiling on 96 randomly chosen isolates of *S.* Tennessee from a variety of sources including human, food, animal, animal feed, and environmental sources.

Descriptive analysis of profiled *S.* Tennessee isolates:

Descriptive epidemiologic data on cases of *S.*Tennessee infections received from participating states department of health were not complete for all variables. Of the 55 profiled human isolates, 11 isolates had missing information for sex. Additionally, 9 isolate samples were missing age information of the patient. Not all isolates had a PFGE pattern listed; therefore, it could not be determined if these isolates were associated with the peanut butter outbreak or not. For each analysis, missing observations for the explanatory variables were automatically deleted by SAS but missing observations are included in the tables for comparison.

Proc freq analysis for the variable source revealed that 55 of the isolates tested were from human, 23 from animal sources, 14 from food items (peanut butter, dry powered eggs), 2 were isolated from environmental sources, 1 from animal feed, and 1 isolate source was unknown. Table 6 displays the frequency counts and percentages of the sources of the profiled *S.* Tennessee isolates. Figure 6 displays a flowchart of the number of isolates profiled and their corresponding sources. Of the 96 isolates profiled, 29 (43.28%) were related to the peanut butter outbreak compared to 38 (56.72%) non-related isolates and 29 isolates were unknown.

Of the 55 profiled human isolates, information for PFGE patterns was listed for 27 (49.91%) isolates. Table 7 displays the PFGE patterns of profiled human isolates in comparison to all profiled isolates. Approximately half of the profiled human isolates had missing information for the variable PFGE. Of the 27 isolates with known PFGE patterns, 16 of the isolates displayed one of the 3 closely related PFGE pattern reported from the outbreak strain by CDC. Of all profiled isolates, 24 of the isolates displayed the 3 closely related PFGE pattern associated with the outbreak.

A total of 23 USDA samples were received of which 22 were from animal sources and 1 was from animal feed. Table 8 is a listing of the distribution of animal sources for profiled *S.* Tennessee animal isolates. Thirty-six percent of the animal samples came from chicks, followed by 27% from cattle. *S.* Tennessee strains from animal sources were non-peanut butter related to the outbreak. All 22 *S.* Tennessee animal isolates displayed Mannose resistant local attachment under microscopic observation. An equal number of profiled isolates (50%, 11/22) were observed to be invasive and highly invasive.

Descriptive epidemiologic analysis of all isolates received:

A total of 194 isolates were received from 5 participating department of community health centers (Michigan, New York, Minnesota, Tennessee, and Indiana), the USDA, and University of Calgary and Penn State. For isolates received from New York, only information such as date of isolation and source of isolate were available, therefore they were removed from the analysis of human isolates. Proc means analysis of 95 human

isolates with available information revealed that the mean age of patients were 52 years of age with a range of 1 year to 94 years, similar to the profiled isolates. Additionally, 81.32% of the human isolates were isolated from patients between 16 years of age and above. Eighty-nine human isolates had information listed for gender, of which 67% were isolated from females compared to 33% for males.

Human isolates:

A total of 55 human isolates were randomly chosen for attachment and invasion of Caco-2 cell models in tissue culture. Age information was available for 46 patients. The mean age of patients was 43 years of age, ranging from 1 year to 94 years. Approximately 36.36% of human isolates were isolated from patients between 16 and 64 years of age and 23.64% were from patients 65 years and over. The most common site of isolation for human isolates was from stool (49.09%). Of the profiled human isolates, cross tabulation table of gender by site of isolation revealed that 49.09% (27/55) percent of human samples tested were from females of which 100% (27/27) were recovered from stool and urine versus 82.35% (14/17) recovered from stool and urine for males (p-value= 0.0152). Table 9 and 10 compares the gender and site distribution of profiled isolates and non-profiled *S.* Tennessee isolates. *S.* Tennessee was more commonly isolated in stool and urine of females than males.

Interaction of *S.* Tennessee with Caco-2 cells:

Ninety-three (97%) of the isolates tested displayed a positive attachment to the Caco-2 cell models maintained in tissue culture medium as recommended by ATCC instruction

for maintaining these cells. There were no differences in attachment patterns of isolates observed between different sources under a light microscope. Additionally, isolates were also tested whether they were sensitive or resistant to D-Mannose used at 1% in the tissue culture medium. Of the 96 isolates tested, 3 were observed to be Mannose sensitive and 93 isolates expressed local attachment in the presence of 1% Mannose (Mannose resistant). Table 11 displays the interaction profiles of tested *S.* Tennessee isolates with corresponding sources. Fifty- eight percent (55/96) of the isolates tested were invasive, compared to 42% (40/96) for highly invasive (Table 11). Comparison of invasion patterns of all profiled *S.* Tennessee strains associated with the outbreak revealed that 75.86% (22/29) were observed to be highly invasive under microscopic examination. Because 97% of the isolates were positive for attachment, therefore we could not assess this variable with exposure variables of interest. Univariable analyses of outcome variable attach with exposure variable of interests revealed insignificant results.

Univariable analysis:

The univariable analysis of outcome invasion with selected predictor variables of interest for profiled *S.* Tennessee human isolates and all profiled *S.* Tennessee isolates are displayed in table 12 and 13. For profiled human isolates, outbreak, age, and gender were found to be insignificant at the 5% alpha value (Table 12). However, for all profiled *S.* Tennessee isolates, it was observed that *S.* Tennessee strains associated with the peanut butter outbreak were more likely to be highly invasive than strains from non-outbreak sources OR= 4.025 (p-value=0.0088, 95% confidence interval (CI): 1.419, 11.418) (Table 13).

Multivariable analysis:

A multivariable analysis was performed to test for possible interactions. All interactions included in the model were found to be insignificant. Age and gender were found to be insignificant in the univariable analysis. Calculation of the percent change in the OR estimates of the covariates age and gender revealed that age is a confounding variable. The percent change in the OR estimate for age was 50.048 %. The percent change in the OR estimate for gender was 0.490%; therefore gender is not a confounding variable. However, gender was also controlled for in the multivariable model (Table 14).

Genomic analysis of *S.* Tennessee isolates:

Sixty *S.* Tennessee isolates were screened by MLST and Multi-locus VNTR Analysis (MLVA). Two genotypes were identified by MLST and 3 were identified by MLVA. Two sequence types (ST) were identified: a major type and minor type. Two non-outbreak strains were identified for the minor type. Sequence variation was observed in dnaN, hisD, and thrA. No sequence variation was observed in the 7 genes in isolates during the outbreak period (August 2006 to June 2007). The neighbor- joining tree of *S.* subspecies enterica based on the 7 MLST genes are displayed in figure 7.

DISCUSSION

The emergence of *S.* Tennessee in peanut butter has caused over 800 cases, perhaps many more. The emergence of *S.* Typhimurium again in 2008-2009 lead to a higher number of cases compared to *S.* Tennessee. The occurrence of the two outbreaks underscores the ability of *S.* Tennessee as a newly emerging foodborne pathogen. In the United States, newly recognized emerging foodborne bacterial pathogens and well recognized pathogens are associated with new food vehicles. Examples of such recognized foodborne pathogens known for causing most severe illness are: *Camplylobacter jejuni, Salmonella, Escherichia coli* O157:H7, *Listeria monocytogenes,* and *Toxoplasma* (56, 57). These foodborne pathogens are foodborne zoonoses, having an animal reservoir from which they spread to humans (58). Additionally, the spread of these foodborne bacterial pathogens are global. Pathogens such as *Salmonella* has spread around the world since the 1980s, whereas *S.* Typhimurium DT 104 is appearing in North America and Europe (58). New foodborne bacterial pathogens are being identified at an increasing rate, suggesting that many more remain to be discovered (58).

The information available on the interaction of *Salmonella* spp. and host cells is limited. There is little knowledge on the mechanism of bacterial attachment, colonization, and invasion (59). The specific determinants involved in these processes have not been fully identified or understood (59). The process of bacterial attachment to human epithelial cells is an essential step for initiation of bacterial infections and bacterial invasion leads to observations of disrupted Caco-2 cell membranes. Furthermore, the adherence of the *Salmonella* spp. to the intestinal cell surface of the host is essential for initiation of

bacterial pathogenicity (59). Attachment and invasion profiling of *S.* Tennessee strains were conducted to as a method to the virulecne of *S.* Tennessee strains associated with the peanut butter outbreak, 2006-2007. Caco-2 cells, when grown to 80% confluency differentiate and resemble columnar epithelial cells (46, 47, 48). The use of Caco-2 cell attachment assays for *S.* Tennessee bacterial strains revealed a high percentage of the tested isolates to express attachment to this type of cell model. Furthermore, in the presence of Mannose, *S.* Tennessee strains were Mannose-resistant as they displayed attachment to Caco-2 cells in the presence of this sugar. The high percentage of the tested isolates that attached to Caco-2 cells suggests that these strains of *Salmonella* enterica serovar Tennessee expresses certain surface structures that plays a role in adhering to Caco-2 cells (59), which may be essential for initiation of an infection. It was also observed that *S.* Tennessee strains associated with the peanut butter outbreak were highly invasive in comparison to isolates from other sources. This observation suggests that *S.* Tennessee strains associated with the outbreak are more likely to be invasive than strains from non-outbreak sources. The high level of attachment and invasiveness observed within the *S.* Tennessee strains associated with the peanut butter outbreak could be due to peanut butter as the source of food vehicle. Peanut butter could have served as a conducive medium enabling the *S.* Tennessee strains to express certain virulence genes involved in these processes.

Descriptive epidemiologic study of clinical cases revealed that a higher number of *S.* Tennessee strains profiled and those not profiled were isolated from females than from males. Similarly, CDC reported a higher percentage of female patients (73%) compared

to males. *S.* Tennessee infections are rare, however, they are more likely than other serotypes to infect the urinary tract (31). Urinary tract infections are common among females. A high proportion of isolates were from the urine, therefore possibly explaining the high number of cases among females in comparison to males.

Salmonella infections are more prevalent among the young, immunocompromised, and elderly. We observed a high number of isolate samples obtained from patients 65 years of age and above. For the young, the number of isolate samples obtained was lower than other age groups. However, information for age was unknown for 9 clinical samples out of the 55 profiled isolate samples. It could be possible that these samples were isolated from those less than 5 years of age, but this remains unknown.

CONCLUSION

All profiled *S.* Tennessee isolates procured from participating health departments all displayed attachment to the Caco-2 cells model grown in tissue culture, an essential initial step leading to invasiveness of the strains. All profiled *S.* Tennessee strains were observed to be invasive; however, *S.* Tennessee strains associated with the peanut butter outbreak were highly invasive compared to invasive isolates from other sources or isolates that were not associated with the outbreak. Food vehicles such as peanut butter, having a high fat and low water content, could play a role serving as a conducive medium for optimal growth and expression of certain surface structures that enhances the strain's attachment and invasiveness as observed in the *S.* Tennessee outbreak strains.

The emergence of known or newly recognized serotypes appear to be a continuous challenging process for health communities as seen in the *S.* Tennessee outbreak and again in *S.* Typhimurium, in which both occurred in a high fat low water content food vehicle. The occurrence of these two outbreaks suggests that our perceptions need to be reviewed from a perception that is based on the fact that food sources such as peanut butter previously thought safe can be considered hazardous. Therefore, consumption of peanut butter can be a risk factor in the etiology of sporadic non-typhoidal *Salmonella* infections among adults and children. It is not sufficient enough to only educate food producers, handlers, and consumers in basic food safety. More in house prevention programs and testing should be sought to mitigate public exposure to emerging contaminated food items and protecting consumers from severe illnesses resulting from foodborne bacterial pathogens.

Food industries should use the occurrence of this outbreak to identify lessons to be learned and develop applicable procedures for use among their industries. An increase in more regular and sensitive testing policies for peanut butter and other food items for *Salmonella*, prior to the release will improve the safety of these food products, prevent distribution of contaminated peanut butter jars and future damaging outbreaks caused by *Salmonella*.

Table 1. Selected *Salmonella* foodborne outbreaks and associated food vehicle(s) [Source: compiled from various CDC-MMWR reports]

Year	Serotype	Outbreak source	Ref
2009	Saintpaul	Raw alfalfa sprouts	*(60, 61)*
2008-2009	Typhimurium	Peanut Butter	*(35)*
2008	Saintpaul	Jalapeno peppers/ raw produce	*(62)*
2008	Litchfield	Cantaloupe	*(63)*
2006-2007	Tennessee	Peanut butter	*(31)*
2007	I 4,5,12:i:-	Frozen pot pies	*(64)*
2006	Typhimurium	Raw tomatoes	*(65)*
2004	Braenderup & Javiana	Roma tomatoes	*(66)*
2003	Enteritidis	Raw almonds	*(67)*
2004	Typhimurium	Ground beef	*(61)*
2002	Newport	Beef	*(68)*
2001	Kottbus	Alfalfa sprouts	*(69)*
2000-2002	Poona	Cantaloupe	*(70)*
2000-2001	Listeriosis	Homemade Mexican-style cheese	*(71)*
1999-2001	Enteritidis	Raw shell eggs	*(72)*
1999	Muenchen	Orange juice	*(73)*
1998	Agona	Toasted oats cereal	*(74)*
1996-1998	Enteritidis	Raw shell eggs	*(75)*
1994-1995	Enteritidis	Raw shell eggs	*(76)*
1994	Typhimurium	Raw ground beef	*(77)*
1994	Enteritidis	Ice cream	*(78)*
1994	Montevideo	Beef jerky	*(79)*
1990-1993	Enteritidis	Ziti (baked) eggs	*(80)*
1993	Tennessee	Powered milk and infant formula	*(32)*
1991	Enteritidis	Raw shell egg	*(81)*
1990	Enteritidis	Bread pudding	*(82)*
1989	Enteritidis	Grade A shell eggs	*(83)*
1981-1982	Dublin	Raw milk	*(84)*

Table 2. *Salmonella* Tennessee infections associated with peanut butter, United States, 2006-2007, case findings [Source: Morb Mortal Wkly Rep. 2007. 56; 521-4]

Variable	%
Gender	
Female	73
Male	27
Symptoms *	
Diarrhea	72
Abdominal cramps	65
Fever	43
Dysuria	45
Source of isolates	
Stool	61
Urine	35
Other specimens	4

*Symptoms onset dates were known for 481 of 628 patients and ranged from August 1, 2006-April 23, 2007

Table 3. Findings from case-control study conducted by state and local health departments [Source: Morb Mortal Wkly Rep. 2007. 56; 521-4]

Variable	Controls (N=124)		Cases (N=65)		Matched Odds Ratio (mOR)	95% Confidence Interval (CI)
	No.	%	No.	%		
Ate peanut butter	82	65	53	81	1.9	(0.8-5.2)
Ate peanut butter more than once a week	50	40	43	66	3.5	(1.4-9.9)
Ate either Peter Pan or Great Value peanut butter	16	13	44	67	10.9	(3.8-43.0)

Table 4. Selected peanut butter and other peanut containing recall products [Source: http://www.accessdata.gov/scripts/peanutbutterrecall/index.cfm]

Product recall category	**Distributor (brand)**
Brownie product recalls	Boston Cookies
	Family Fresh Markets
	Food Bonanza
Cake and pie product recalls	Awrey Bakeries
	Food 4 Less
	Kroger
Candy product recalls	Buffalo Chips
	Brach's
	Fannie May
Cereal product recalls	Nature's Path
	Bear Naked
	Michaelene's
Cracker product recalls	Meijer
	Keebler
	Little Debbie
Dressing and seasoning product recalls	Kariba Farms
	WOW
Fruit and vegetable product recalls	Eating Right
	Nutty Nanners
	Trader's Joe
Ice cream product recalls	#216 Schwan's
	Shop 'n Save
	Sysco
Peanut product recalls	Marker Pantry
	Piggly Wiggly
	Peanut Corporation of America
Peanut butter product recalls	King Nut
	Town & Country
	Peanut Corporation of America
Peanut paste product recalls	Peanut Corporation of America or Parnell's Pride
Pet food product recalls	Breadfarrm
	American Nutrition, Inc.
	Next Gen Pet Products
Pre-packaged meals product recalls	Dinners ready
	Reser's
	Ethnic Gourmet
Snack bar product recalls	Special K Protein
	Nutrisystem
	Kashi TLC
Topping product recalls	Barefoot Contessa
	Best Choice

Table 5. *S.* enterica subsp. Enterica – 7 housekeeping genes (MLST *Salmonella* database)

Gene	Protein	Size (bp)
*hem*D	Chorismate synthase	432
*dna*N	DNA polymerase III, beta-subunit	501
*thr*A	Uroporphyrinogen III synthase	501
Pure	Histidinal dehydrogenase	399
*suc*A	Phosphoribosylaminoimodazole carboxylase	501
*aro*C	2-oxoglutarate dehydrogenase decarboxylase component	501
*his*D	Aspartokinase I	501

Table 6. Source frequency counts and percents of profiled isolates

Source	No.	%
Human	55	57.30
Animal	23	23.96
Food	14	14.58
Feed	1	1.04
Environmental	2	2.08
Missing	1*	1.04
Total	96	100

*1 *S.* Tennessee isolate source was unknown

Table 7. PFGE patterns of profiled human *S.* Tennessee isolates compared to all profiled *S.* Tennessee isolates

PFGE patterns associated with *S.* Tennessee outbreak	**Profiled human isolates**		**All profiled isolates**	
	No.	**%**	**No.**	**%**
JNXX01.0010	3	5.45	4	4.17
JNXX01.0011	9	16.36	16	16.67
JNXX01.0026	4	7.27	4	4.17
Other PFGE patterns				
JNXX01.0001	3	5.45	4	4.17
JNXX01.0002	1	1.82	1	1.04
JNXX01.0012	2	3.64	2	2.08
JNXX01.0014	1	1.82	3	3.13
JNXX01.0030	1	1.82	1	1.04
JNXX01.0039	1	1.82	1	1.04
JNXX01.0049	2	3.64	2	2.08
Missing PFGE	28	50.91	58	60.42
Total	55	100	96	100

Table 8. Frequency counts and percentages of animal sources from *S.* Tennessee isolates received

Animal type	**No.**	**%**
Alphaca	1	4.55
Avian	2	9.09
Cattle	6	27.27
Chick	8	36.36
Goat	1	4.55
Swine	3	13.64
Turkey	1	4.55
Total	22	100

Table 9. Gender and site distribution of profiled *S.* Tennessee from human source

Gender	**Profiled human isolates N=55**		**Site of isolation for profiled isolates from human sources**							
	No.	**%**	**Stool**		**Urine**		**Other**		**Missing**	
			No.	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**
Female	27	49.09	15	53.85	12	46.15	0	0	0	0
Male	17	30.91	12	70.59	2	11.76	3	17.64	0	0
Missing	11	20.00								
Total	55	100								

Table 10. Gender and site distribution of all human isolates received

Gender	Profiled human isolates N=95		Site of isolation for profiled isolates							
	No.	%	Stool		Urine		Other		Missing	
			No.	%	No.	%	No.	%	No.	%
Female	60	63.16	31	55.36	25	44.64	0	0	4	6.7
Male	29	30.53	20	76.92	4	15.38	2	7.69	3	10.34
Missing	6	6.31								
Total	95	100								

Table 11. Virulence profiling of profiled [1] *S.* Tennessee isolates with sources

Interaction Profiles	Sources					
	Human	Animal	Food	Feed	Environmental	Total*
Attachment positive	53 55.79%	23 24.21%	13 13.68%	1 1.05%	2 2.11%	92 96.84
Attachment negative	2 2.11%	0 0%	1 1.05%	0 0%	0 0%	3 3.16%
Highly invasive	34 35.79%	11 11.58%	9 9.47%	0 0%	1 1.05%	55 57.89%
Invasive	21 22.11%	12 12.63%	5 5.26%	1 1.05%	1 1.05%	40 42.11%

*A total of 96 *S.* Tennessee isolates were profiled, 1 isolate source was unknown

[1] Profiled isolates are *S.* Tennessee isolates chosen randomly for virulence profiling of attachment and invasion pattern using Caco-2 cells model grown in tissue culture. *S.* Tennessee isolates where virulence profiling was not performed were classified as non-profiled.

Table 12. Univariable analyses of outcome highly invasive with selected predictor variables for profiled human *S.* Tennessee isolates

Variables	Odds Ratio	95% CI	P-value	Missing
Outbreak	4.444	(0.803, 24.609)	0.0876	11
Gender	0.521	(0.143, 1.893)	0.3218	25
Age:				9
<5 years	1.333	(0.196, 9.083)	0.7689	
=5 and <16 years	1.666	(0.257, 10.789	0.5921	
=65 year	0.778	(0.190, 3.187)	0.7269	
Site:				6
urine	0.643	(0.185, 2.234)	0.4870	
other	0.225	(0.018, 2.814	0.2472	

* Male was used as a reference group for gender
Age group of =16 and <65 was used as a reference group for age
Stool was used as a reference group for site (other consists of blood and wound)

Table 13. Univariable analysis of outcome highly invasive with variable outbreak for all profiled *S.* Tennessee isolates

Variable	Odds Ratio	95% CI	P-value	Missing
Outbreak	4.025	(1.419, 11.418)	0.0088	29

Table 14. Multivariable analysis of peanut butter exposure controlling for age and gender

Variables	Odds Ratio	95% CI	P-value	Missing
Outbreak	4.668	(0.491, 44.364)	0.180	11
Gender	0.980	(0.084, 11.384)	0.987	25
Age:				9
<5 years	0.212	(0.085, 52.412)	0.648	
=5 and <16 years	0.339	(0.027, 4.178)	0.399	
=65 year	0.913	(0.083, 10.014)	0.940	

* Male was used as a reference group for gender
Age group of =16 and <65 was used as a reference group for age

Figure 1. The pathogenic spectrum for *Salmonella* infections (12, 13)

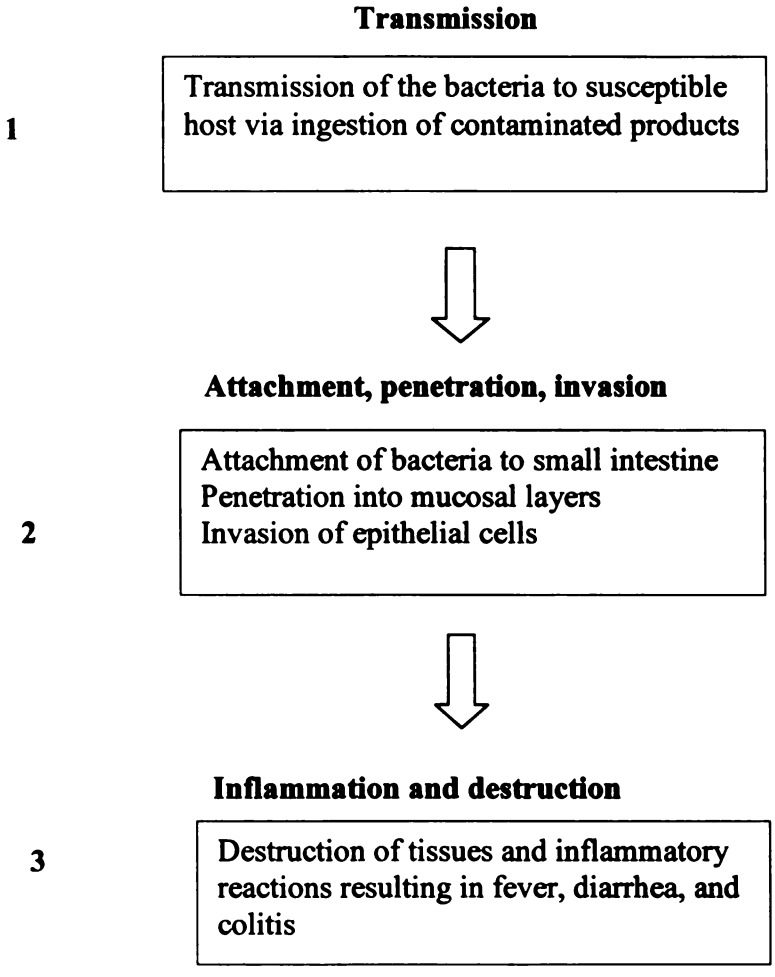


Figure 2. Reported cases of salmonellosis, United States, 1976-1999
[Source: Morb Mortal Wkly Rep. 2009. 56; 79-85]

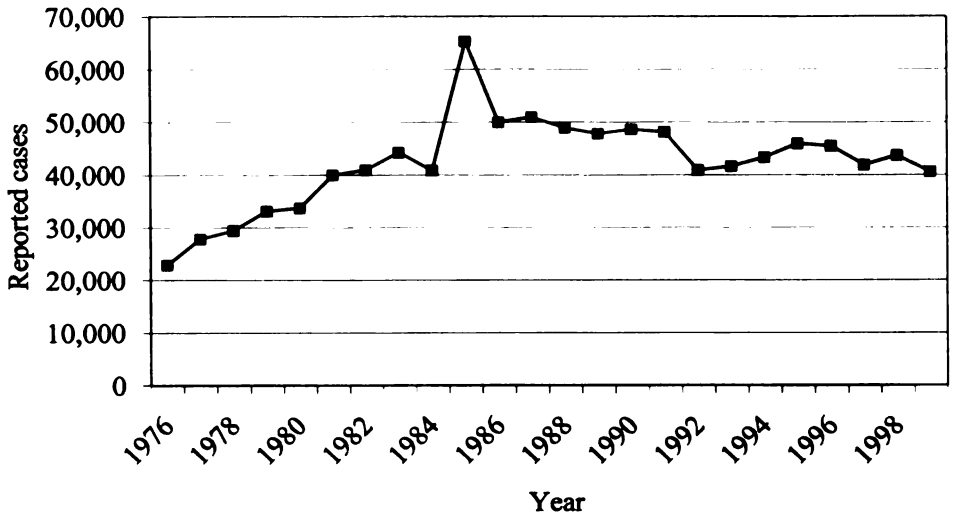


Figure 3. Reported incidence of salmonellosis, United States, 1997-2007
[Source: Morb Mortal Wkly Rep. 2009. 56; 79-85]

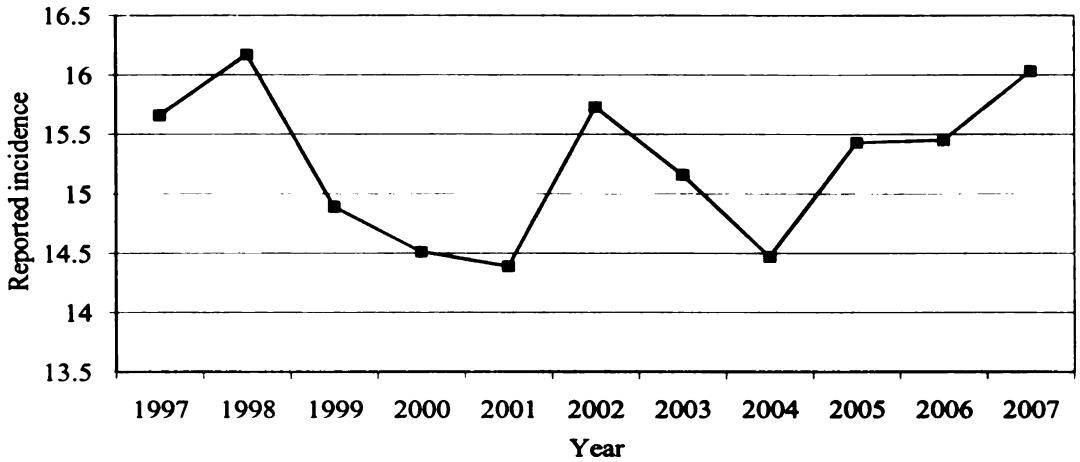


Figure 4. *Salmonella* strains of other serotypes found in food and in environmental samples during *Salmonella* Typhimurium outbreak investigation*
[Source: http://www.cdc.gov/salmonella/typhimurium/strains_table.html]

Location of sample origination	**Other *Salmonella* serotypes**	**DNA fingerprint ID (PFGE pattern)**	**PFGE image**	**Source in which strain was found**
Georgia	Mbandaka	TDRX01.0011		PCA plant in Blakely, Georgia-floor crack
Georgia	Senftenberg	JMPX01.0049		PCA plant in Blakely, Georgia-floor crack
Georgia	Tennessee	JNXX01.0011		Unopened container of King Nut brand peanut butter
Minnesota	Tennessee	JNXX01.0026		Unopened container of King Nut brand peanut butter

*These strains are not associated with an increase in human illness

Figure 5. Multi Locus Sequence Typing (MLST): 7 housekeeping genes (*Salmonella* MLST database)

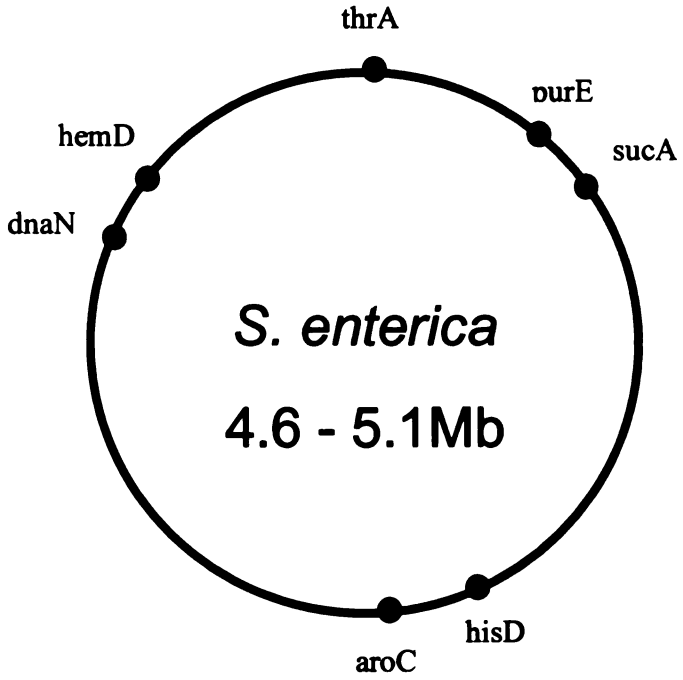


Figure 6. Sources of profiled *S.* Tennessee isolates

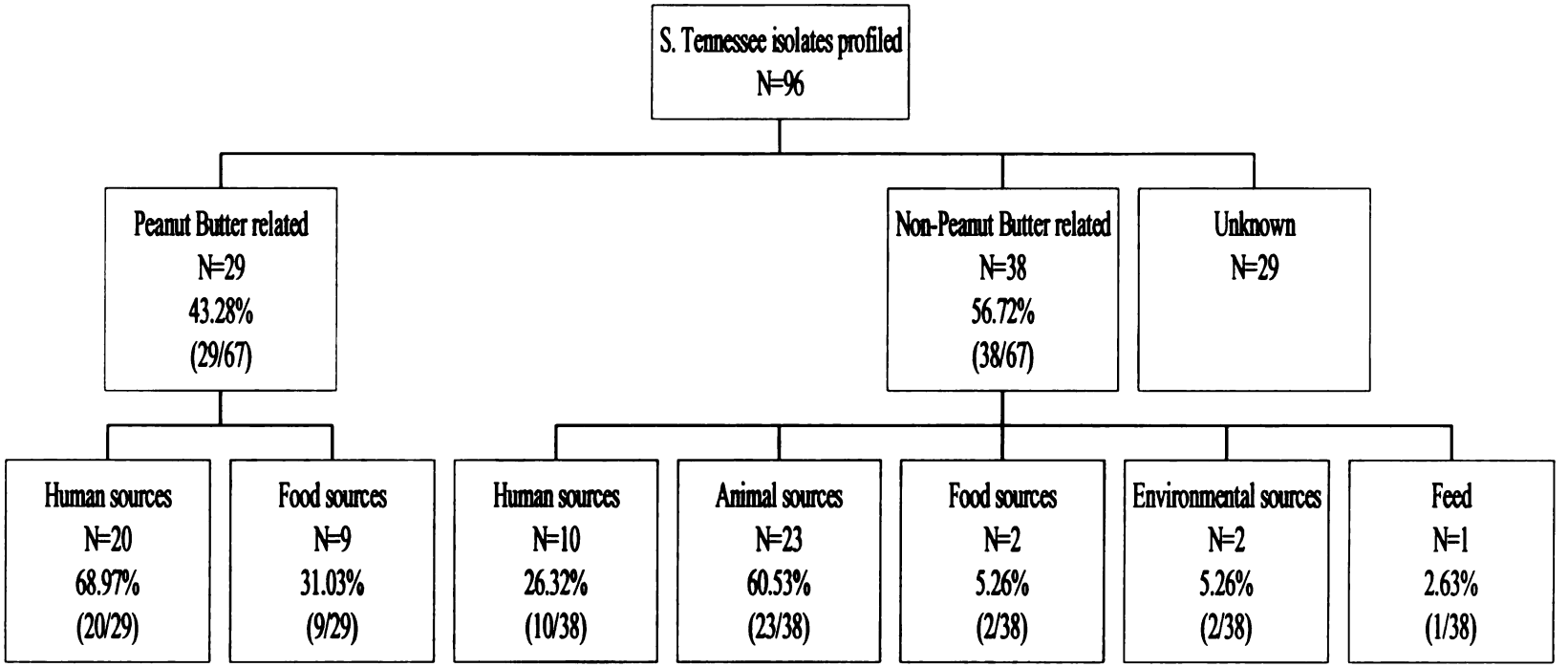
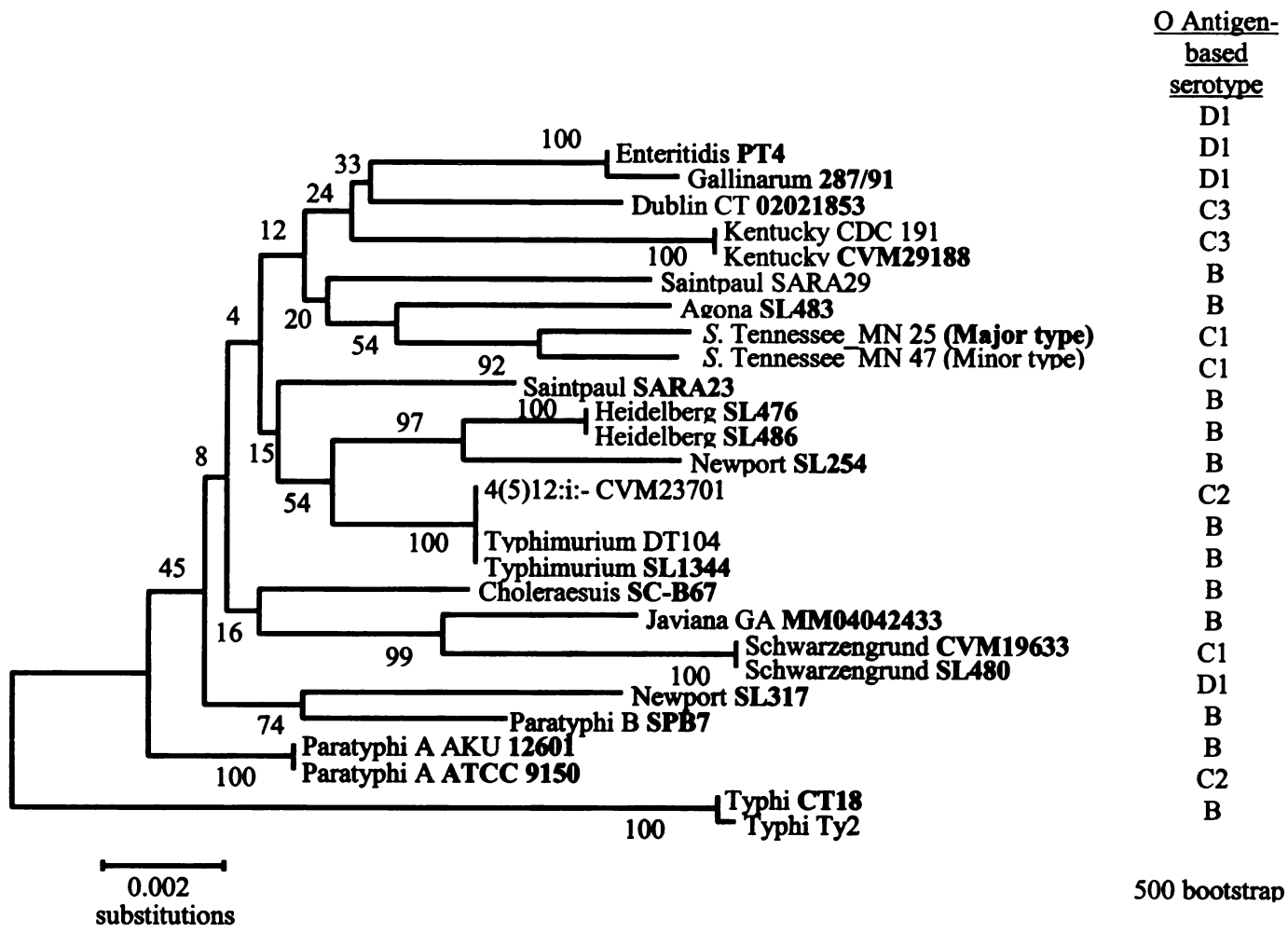


Figure 7. Neighbor-Joining tree of *S. subsp.* Enterica based on 7 MLST genes



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