SPATIAL AND TEMPORAL ANALYSIS OF *SALMONELLA ENTERITIDIS* OUTBREAKS IN USA, 1990-2015

By

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ABSTRACT

SPATIAL AND TEMPORAL ANALYSIS OF *SALMONELLA ENTERITIDIS* OUTBREAKS IN USA, 1990-2015

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To address an endemicity of *Salmonella Enteritidis* (SE) infection, a food borne disease, in United States of America (USA), different food vehicles associated with SE outbreaks were examined in this study. Data of all SE outbreaks reported to Centers for Disease Control and Prevention (CDC) from 1990 to 2015 was retrieved from CDC website. The data included following information about each outbreak: year, month, state, implicated food vehicles, location, and number of SE cases. It was found that eggs-based dishes 273 (24%) were the highest reported followed by other implicated food items; meat 130 (11%), vegetables 96 (8%), chicken items 95 (8%), dairy products 55 (5%), and bakery items 8 (1%) in the country. Relative occurrence of food vehicles compared to eggs-based dishes implicated in SE outbreaks was examined by using negative binomial model which showed significant contribution of other food items in causing SE outbreaks in the country such as meat ($\exp(\beta)=0.51$, 95% CI 0.37, 0.69), chicken ($\exp(\beta)=0.42$, 95% CI 0.30, 0.58), vegetables ($\exp(\beta)=0.41$, 95% CI 0.29, 0.55), and dairy items ($\exp(\beta)=0.27$, 95% CI 0.18, 0.40). In addition, different trends of SE outbreaks were analyzed based on available dataset, and newly created categorical variables such as census region, HHS regions and seasons. The study enhanced the existed knowledge of other implicated food items besides eggs in persistent occurrence of SE food borne disease in the USA.

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KEY TO ABBREVIATIONS

SE	*Salmonella Enteritidis*
CDC	Centers for Disease Control and Prevention
HHS	Human and Health Services
CI	Confidence Interval
MOR	Marginal Odds Ratio
WHO	World Health Organization
USA	United States of America
USDA	United States Department of Agriculture
SEPP	SE Pilot Project
PEQAP	Pennsylvania Egg Quality Assurance Program
FDA	Food and Drug Administration

CHAPTER 1: INTRODUCTION

Salmonella serotypes are the leading causes of diarrhea and gastroenteritis in the world. It has been estimated that 93.8 million of cases of nontyphoidal salmonellosis and 155,000 deaths occur every year in the world where 86 percent of all these illnesses are due to foodborne infections. Nontyphoidal *Salmonella* serotypes are a major cause of foodborne infections and outbreaks in the world (1). Increasing foodborne infections have become serious public health issue in both developing and developed countries. A study on estimating the burden of foodborne diseases in the USA was conducted by using active and passive surveillance data of 31 different food pathogens. It was estimated that every year, about 9.4 million foodborne infections occur in the USA. The study results showed that nontyphoidal *Salmonella* caused over 1 million infections (28%) out of 3.6 million bacterial infections in the USA. It was reported that nontyphoidal salmonellae are a major cause of foodborne diseases in the USA (2). Among nontyphoidal *Salmonella* serotypes, *Salmonella* Enteritidis (SE) is the most commonly reported serotype. Based on isolates of *Salmonella* Enterica collected from human laboratories of 37 countries from all over the world (2001-2007), *Salmonella* Enteritidis (SE) was ranked first with around 43.5% proportion of all *Salmonella* isolates. SE ranked first among Nontyphoidal *Salmonella* infections among humans in the USA (3). In a recent study, it was reported that 38 % of reported nontyphoidal Salmonella outbreaks were caused by SE from 1973 to 2009 in the USA (4). Similarly, it was estimated that SE isolates represent 13-19% of overall *Salmonella* isolates in the USA (4,5). Another recent study also showed an increasing trend of SE isolates per 100,000 population over *Salmonella* Typhimurium serovar, a previous highly prevalent *Salmonella* serotype in the USA (6). These findings clearly prove SE as a major *Salmonella* serotype associated with outbreaks of foodborne *Salmonella* infections. More importantly, a

study on genome comparison of different Salmonella enterica serovars showed that SE has gained such diversity both genomic and metabolically that it can survive and infect multiple hosts (7). Persistent occurrence of SE outbreaks across the country requires epidemiological studies to identify important risk factors and routes of disease transmission to address this public health challenge in the USA. In addition, Frenzen et al. showed that salmonellosis has a significant economic impact in the USA, it overall costs 0.5$ to 2.3$ billion yearly and SE is one of the major *Salmonella* serotypes, ranked one, in causing Salmonellosis in the country (8).

In the past, few studies were designed to address food sources associated with SE outbreaks in US. Those studies showed that raw and uncooked eggs products were the main source of SE outbreaks in the country. Implementations of different guidelines regarding eggs' handling, transportation and storage led to a tremendous decline in eggs associated outbreaks. However, there are still significant SE outbreaks occurring in the country yearly which can be due to consumption of other SE-contaminated foods that have not been addressed in previous studies. Considering the above factors, this study is designed to elucidate the role of different foods associated with SE outbreaks at various time points and places in the country.

CHAPTER 2: BACKGROUND

Numerous studies found that diarrhea is among the most common health issues in developed countries including Australia, Canada and USA. Diarrhea was more likely reported in females and children under the age of five years (9). It shows the importance of recognizing food borne diseases, which in most cases, cause diarrhea in humans. There are multiple bacterial, viral and parasitic causes of food borne illness, however, Salmonellosis is a major cause of diarrhea in the country. This was found from a study which was conducted using data from both active and passive surveillance of food borne infections caused by thirty-one pathogens from 2000 to 2006 in the USA (10). It was found that non typhoidal *Salmonella* serotypes are the leading cause of hospitalization (35%) and death (28%) among all foodborne infections that occurred in the country during 2000 to 2006 (10). A study has been conducted on *Salmonella enterica* serotypes-based outbreaks and associated food items reported to CDC from 1998-2008 in USA found that among single food implicated-outbreaks, SE caused more outbreaks of approximately 36% (144/403) followed by Typhimurium (14%) Newport (10%) and Heidelberg (6%). It revealed that SE is causing more foodborne outbreaks than any other serotype of Salmonella enterica in the USA (11). According to an annual report of food borne disease outbreaks in USA, 2015, it was found that Salmonella is second common cause of single-etiology confirmed outbreaks in the country, causing 149 of 920 (34%) outbreaks, and 3944 (39%) cases following Norovirus which caused 164 of 920 (37%) outbreaks, and 3893 (39%) cases. Among 164 Salmonella outbreaks, SE was the most common serotype and was reported in 51 outbreaks (35%) (12).

2.1 Eggs

SE infection has been the most prevalent type of salmonellosis among humans in the US for many years. It is a food borne infection and spreads through consumption of different SE-contaminated food vehicles. In the past, eggs-containing products had been significantly associated with SE outbreaks in the USA. The data from National Surveillance Sources (1973 to 1984) showed that around 44% SE outbreaks were associated with egg-containing products. Those outbreaks were associated with eggs which were neither contaminated by farm soil nor were cracked. The first study conducted by St Louis et al. revealed that 27 (77%) of the 35 SE outbreaks, occurring during 1985-1987, were associated with Grade A shell eggs (13). Later, shell eggs-associated SE outbreaks raised the possibility of transovarial transmission of SE bacteria in egg laying chickens (14). In addition, under the National Poultry Improvement Plan (NPIP), SE was identified in diverse sampling from thirty-six environmental, six non-viable fertilized eggs, and fifteen birds from sanitation-monitored breeder flocks since 1990 [A.R. Rhorer, unpublished data, (15)]. Moreover, it has been recorded that the prevalence of different PT of SE changed over time such as in the 1980s, the most prevalent PT of SE were PT13a and PT8 (16) while in early 1990, PT23 and PT8 were the most common causes of SE associated human outbreaks in the country (17). After that, an emergence of PT4 occurred in the US when first PT 4 associated SE outbreak was reported in Texas state in 1993 followed by PT4 outbreaks in Utah and California. The study showed that the emergence of PT4 in the west region caused more outbreaks than above-mentioned phage types (PT8, PT13a, PT23) in the country (18). For example, it was estimated that 496 cases of SE were recorded in Los Angeles County, California in just four months (April to July) in 1994 (19). All these findings projected a great concern for

public health department because the infected birds can spread the disease in flock quickly and can cause the disease outbreaks among humans by contaminated eggs.

After recognition of shell eggs as a potential source of SE outbreaks, traceback program was introduced by United States Department of Agriculture (USDA) to find out the outbreak's source and origin. Altekruse *et al*. conducted a study under the traceback program and found that SE phage types associated with human outbreaks were recovered from 100% and 88% of the environmental samples and internal organs of hens respectively from fourteen different implicated poultry farms (17). It shows that traceback program was helpful in tracing and confirming the source or origin of SE outbreaks among humans which were caused by contaminated eggs (17). SE became the most frequently reported *Salmonella* serotype in 1990 in the USA. It accounted for 21% of all salmonellosis in 1990. From 1985-91, there were 380 outbreaks (213 food vehicle-unconfirmed and 167 foods vehicle-confirmed). Of 167 food vehicle-confirmed SE outbreaks, 137 (82%) were egg-associated. There were 59 SE outbreaks reported in hospitals and nursing homes accounted for most of the deaths in this study period. Thirteen out of fifty nine also found eggs associated SE outbreaks (20).

Similarly, Patrick et al. studied SE outbreaks reported to CDC from 1985-1999 in the USA (21). There were 841 outbreaks reported to CDC and only 371 outbreaks reports had information about food vehicle and they found that 298 (80%) were egg-associated outbreaks. These eggs- associated outbreaks were associated with very diverse egg-containing recipes such as raw egg dishes (egg-nog, tiramisu, Caesar salad dressing), lightly cooked egg-dishes (hollandaise, sauce, meringue) cooked egg dishes (lasagna, ziti, and stuffing) and egg battered dishes (chiles rellenos, egg rolls etc.) It shows that egg-containing recipes cooked in several

styles were equally capable to cause SE infections in humans. It might suggest the occurrence of contamination while cooking, serving or any other raw food mixing with egg-containing dishes. Next, they found that of 371 SE outbreaks, 20 (27%) outbreaks were chicken or turkey, 8 (11%) beef, 6 (8%) shrimps, and 9 (13%) vegetables associated. It shows that SE outbreaks happened from 1985-1999 were mostly egg associated which matches other studies and ours as well (21).

After identification of high numbers of eggs-based SE outbreaks in 1990, vigorous preventive measures were advised and implemented in the country (15). In February 1990, USDA implemented SE regulation after recognizing a highly increased number of SE outbreaks and cases. Mostly these outbreaks occurred in the north-east region of the country. Traceback program was introduced by USDA to find the origin of contaminated eggs associated with SE outbreaks in humans. After identifying implicated poultry farms, SE presence was tested in the environment and laying hens. In addition, a survey was conducted by USDA in 1991 and 1995 to detect the SE prevalence in consumed hens at slaughterhouses and unpasteurized eggs at breaker-plants, and result of this study showed an increased prevalence of SE in all across the country (15,22). Later, the USDA started a quality assurance program, SE Pilot Project (SEPP), in 1992 by a collaboration of research institutions, University of Pennsylvania, Pennsylvania State University, and Pennsylvania Department of Agriculture, and Pennsylvania Poultry Federation. Under the SEPP program, samples from birds, rodents, and the environment from poultry farms were tested. If farms were SE positive, then eggs were tested, and if eggs were also SE positive then all eggs from that farm were sent to pasteurization plant before sending them to markets. Later, in 1994, the SEPP was changed into Pennsylvania Egg Quality Assurance Program (PEQAP) (15). In 1995, an environmental survey was conducted and found that layer farms participated in SEPP or PEQAP program had reduced infections and isolation of SE (23).

Another study showed that the diversion of eggs from farm to pasteurization plant under the SEPP program resulted in a remarkable reduction of human SE cases and outbreaks in the north-east region (24). It shows that overall PEQAP program played a key role in controlling of SE infections and outbreaks in the north-east region. Similarly, other major step taken by governmental agencies and eggs producing and storage companies was storage of eggs at efficient temperature to further reduce SE outbreaks. It was estimated in 1998 that the refrigeration of eggs after laying, packing, and shipping could reduce SE cases by 8% and 12% respectively (25), and in August 1998, the USDA issued regulations of eggs storage and transportation at temperature no higher than 45°F (7.2°C) and labeling of containers with storage instructions (26). Moreover, in 1999, Seventeen different states implemented a rule to print an expiry or "sell-by" date on egg packs (27). Next, Food and Drug Administration (FDA) also issued a regulation in 2000 which requires proper refrigeration of eggs for sale at retail stores (28). To control the SE illnesses, the President's Council on Food Safety declared an Egg Safety Action Plan on December 10, 1999, stating the goal to reduce eggs associated SE infections by half by 2005 and eliminate them by 2010 (29). In brief, multiple preventive measures against eggs associated SE outbreaks were taken since the early 1990s to reduce the disease outbreaks. In general, the preventive steps included consumer and producer awareness about the disease, traceback policy, on-farm testing, PEQAP, disease education to vulnerable population such as children, elderly, and pregnant women to avoid consuming raw and uncooked products of eggs. In addition, restaurant and catering places were informed to make sure cleanliness, proper storage, cooking and serving of eggs containing products.

Although many successful interventions were implemented to reduce the eggs associated SE infections and outbreaks, there are still SE outbreaks occurring at a similar rate in the USA.

2.2 Chicken

A case-control study conducted at FoodNet sites showed that chicken was also a major risk factor for SE infections in the USA. (30) USDA survey based on Hazard Analysis and Critical Control Points (HACCP) determined that SE was found in 2.4% of broilers at slaughterhouse (31).

Kimura et al. found that people who ate chicken at dining places out of the home were more likely to have SE infection (MOR: 2.2, 95% CI, 1.4–3.4) than people those ate chicken at home (0.4; 95% CI, 0.3–0.6). It shows that ready to go or ready to eat products of chicken or any other food can be a potential source of SE outbreak occurrence (30).

In studies from the early 1990s, chicken was not a common source of SE infection in the USA. However, it was the endemic and frequent cause of human illness spread through SE infected and contaminated broiler chickens across the world, especially European countries (32,33). Later, specifically, after the emergence of SE PT4 in the USA, there is a shift in the route of SE infection to humans. Currently more outbreaks are occurring due to contaminated chickens instead of eggs, which were a common source in the early 1990s in the USA. Similarly, a study conducted in Thailand (1995) showed that PT4 was a frequent source of chicken contamination in retail places. They identified PT4 among 17 (74%) out of 23 SE isolates collected from retail chicken samples. It is well known from previous and current studies that overall salmonella is more likely to infect chickens, contaminate chicken and meat carcasses and cause salmonellosis in humans as well. Similarly, SE can cause human illness by contaminating chicken and meat carcasses along with eggs (34,35). Roberts et al. (36) reported that SE PT4 was isolated from frozen chicken samples collected under the United Kingdom retails survey in 1990. It means that PT4 contaminated chicken was a significant source of SE infections in Europe

while eggs were major risk factor of SE infections in the USA. It is also possible that PT4 contaminated chicken might have become now a potential source of chicken-based outbreaks in the USA, which has been increasing since 2009 according to our study results. It suggests that phage typing of current circulating serotypes of SE and contaminated chicken might explain more about PT4 specific outbreaks and infections in the USA. However, From a study, the virulence of PT4 in chicks was found significantly higher than other phage types (PT8, PT13a, PT14b) (37).

Similarly, it was reported in an annual report of foodborne outbreaks of 2015 that chicken was implicated in 22 outbreaks (11%). Of 22 chicken outbreaks, salmonella serotypes were implicated in 9 outbreaks which clearly shows that chicken is major source of Salmonellosis (12). A recent source attribution study from Ahlstrom et al (38) showed that chicken was a prominent source associated with salmonella human cases in Minnesota State. In addition, they found that among the salmonella serotypes, SE was a major serotype in causing human cases throughout the study period (2005-2014). It can be inferred from the study that chicken and its products could be a main source of SE infections among humans in Minnesota and the whole country as well.

2.3 Vegetables

Vegetables have been an integral part of many ready to eat products. In addition, fashion of eating raw vegetables in the form of salads, burgers and sandwiches is growing a lot all over the world. Food poisoning, food borne illness and outbreaks has also been increasing but vegetables as major food vehicle associated with SE outbreaks has not been addressed in US. A Mexican study has shown an isolation of SE and other *Salmonella* serotypes from samples of vegetables (39). Another study was conducted in Malaysia to determine the prevalence of

different *Salmonella* serotypes in vegetarian patties, burgers and salads. Authors et al. found the highest numbers of SE positive samples (35/175) among all *Salmonella* serotypes followed by *Salmonella* Typhimurium (25/175). Mostly SE positive samples were lettuce, cabbage and leafy vegetables which might be due to high chance of *Salmonella* attachment on these vegetables' surface (40) Similarly, a study conducted an analysis on leafy vegetables associated with food borne outbreaks in USA from 1973 to 2012. They found out that Salmonella is the third main cause of leafy vegetables associated foodborne outbreaks in the US after Norovirus, and *E. coli.* Salmonella was confirmed or suspected in 10/162 of single leafy vegetables and in 22/444 of leafy vegetables-based salads implicated in food borne outbreaks from 1973 to 2012 (41). According to annual report of food borne diseases outbreaks in USA, 2015. It was found that the most illnesses, 1504, were associated with overall vegetables-based outbreaks (12). Of 1504 illnesses, 1311 infections were caused by Salmonella serotypes which shows that contaminated vegetables are also major source of salmonellosis (12). Moreover, pathogens contaminated vegetables can spread food borne diseases easily and cause more illness than other food items.

Improved methods and regulations of vegetables' packaging and storage can be an important way to reduce the SE survival and growth on vegetables. A study showed a decreasing trend in growth of SE on vegetables stored at 4 °C both in air and in 4.9% CO_2, 2.1% O_2, 93% N_2 conditions (42). However, it has been observed that Salmonella spp. can survive at wide range of temperatures such as from lowest temperature (4 to -8 °C) to highest temperature (44 °C) (43). In addition, proper cooking of vegetables can play a key role in reducing vegetables-associated SE outbreaks and infections.

2.4 Specific Aims

The main goal of this study is to investigate the numbers, spatial and temporal distributions of foods associated with SE outbreaks in the USA (1990-2015) and how they vary by locations, season and regions. To achieve this goal, two specific aims have been made for the study:

2.4.1 Aim 1:

To characterize the foods associated with SE outbreaks in the USA (1990-2015). In previous studies, only eggs' and chickens' products have been analyzed; therefore, this study will explore other sources of foods such as meat products, fresh produce and dairy products etc. to uncover key factors relating to SE outbreaks that may be related to these unreported disease-causing sources. *I hypothesize that fresh produce, vegetable containing dishes and meat products are playing a significant role in causing SE outbreaks and maintaining the disease endemicity in the country.*

2.4.2 Aim 2:

To study locations, seasons, and regions associated with SE outbreaks overall and for different food sources. *I hypothesize that SE contaminated foods sources vary across outbreaks' location, season, and regions.*

CHAPTER 3: DATA and METHODS

3.1 Study Population

Foodborne outbreaks occurred in USA has been regularly reported to Centre for Disease Control and Prevention (CDC) since 1973 (44). It is defined by CDC that occurrence of two or more cases of foodborne disease from same food vehicle will be termed a foodborne disease outbreak (44,45). Reports of these foodborne outbreaks contain information on etiological agents, cases, deaths, hospitalization, year, month, state, food and location. All this information has been posted on CDC website and is publicly accessible. In addition, the data available on CDC website is fully decoded and in summarized form and does not include such an information which can be used to identify and contact subjects. Current study includes all reported outbreaks of SE in USA from 1990 to 2015. Data was retrieved from CDC Food Tool, an online data source of reported foodborne outbreaks in USA from 1998 onward. Data before 1998 was collected from papers-based line-listings of reported foodborne outbreaks available on CDC website. Data was verified from available online sources, organized, and transformed into SAS dataset by recoding the following variables: location, season and food vehicle into categorical variables with various levels.

3.2 Food Vehicles

All food vehicles involved in SE outbreaks are divided into the following 7 categories (Egg-based dishes, Chicken containing dishes, Meat/Seafood containing products, Vegetables, Dairy products, Bakery items, Unknown food). These categories are chosen and made based on the knowledge of primary ingredients of recipes and already known foods reported in previous SE outbreaks. It is already known that eggs and chickens are the main risk factors of SE outbreaks in US but in this study, we want to know about the critical role of other food vehicles,

vegetables, meat and dairy products in endemicity of SE disease in USA. For this purpose, all the plant origin products, vegetables, sprouts and fresh produce are included in category of 'vegetables'. All recipes largely containing milk, cheese, ice cream, butter etc. are included in to category of 'Dairy items'. Bakery origin products such as bread, tortilla, chips etc. are categorized separately 'Bakery items'. Sea food and all types of meat products except chicken are grouped into a category of "Meat Products". It is aimed that this classification will provide valuable information about different other risk factors of SE parallel to Eggs and Chickens containing dishes.

There was much overlap between food categories where we found eggs with meat, or chicken etc. It is a challenge to divide the food vehicles into distinct categories. For example, from initial classification of food vehicle, a table is generated below. In this table, it is attempted to consider all combinations of different food categories into the classification, but it would be hard to run and interpret statistical analysis by inputting above mentioned food vehicles into statistical model. Here, a strategy is designed to classify the food vehicles for the purpose and analysis of the study. Based on the known risk of food associated with SE outbreaks from previous literature, all food categories are ordered in the following way:

eggs items > chicken items > meat items > vegetables > dairy items > bakery. In this way, if eggs are found as an ingredient of outbreak associated food, then the outbreak is considered eggs associated by ignoring the presence of other foods. Similarly, if eggs are absent and chicken is present with other foods then outbreak is assumed to be associated with chicken. Most important, meat and vegetables categories would not be including any eggs and chicken containing dishes. Even in the analysis, we might get less value estimate, but it can be reported and compared with eggs and chicken, known risk factors, rationally which will truly fulfil the study's objectives.

Thus, all food vehicles are divided into seven categories (Egg-based dishes, Chicken containing dishes, Meat/Seafood containing products, Vegetables, Dairy products, Bakery items, and Unknown food).

3.3 Season

Furthermore, based on month of outbreaks and meteorological classification of season, a categorical variable, season: summer, spring, autumn and winter, was created.

3.4 Location

Based on previously reported studies on SE and other foodborne outbreaks, all locations of occurrence mentioned in the data set are categorized into 7 types (Food party, Grocery stores, Nursing/Hospital, Restaurant, School/Prison, Private residence, Unknown place) by considering importantly locations of food preparation, distribution and serving methods. (46)

3.5 Regions

It is also attempted to find any trend of SE outbreaks among different regions of US. Based on given information of state in the data set and Census Bureau's regional classification, we created a categorical variable, census region, with four different regions: Northeast, South, Midwest and West. Similarly, based on regional classification of Human and Health Services (HHS), we also created a categorical variable, HHS, with ten different regions of USA. These regional variables will provide a spatial trend of SE outbreaks' occurrence across the country.

3.6 Group of Years

Knowing the year of outbreaks, we divided twenty-six years (1990-2015) into five groups: 1990-1994, 1995-1999, 2000-2004, 2005-2009, 2010-2015. From literature, it is seen that mostly interventions regarding the control of eggs associated outbreaks were done in 1990-

1995 and 1995-1999. This group classification will not only help to analyze temporal change in overall occurrence of SE outbreaks but also learn about trends of specifically eggs associated outbreaks and impact of related interventions.

3.7 Analytic Plan

First, descriptive statistics (means, standard deviations, frequency and proportions) were calculated for all variables of interest. For statistical analysis, Poisson regression model and Negative binomial model were applied using SAS software (9.4 version) to measure an association between the number of reported outbreaks with different categorical variables (food vehicle, location, season, HHS and census regions).

All geographic analysis and mapping procedures were performed in ArcGIS software (10.5 version).

The following equations of Poisson regression were used to calculate estimates:

$$Log\left(E(Y|X)\right) = \beta_0 + \beta_1 X_1 + \cdots + \beta_p X_p$$

$$\left(E(Y|X)\right) = e^{\beta_0 + \beta_1 X_1 + \cdots + \beta_p X_p}$$

Where $X_1,\ldots,X_p$ are terms for categorical variables (covariates). A p-value of < 0.05 was used to declare statistical significance of covariates.

CHAPTER 4: RESULTS

4.1 Food Categories Associated with SE Outbreaks

There were 1144 single-state SE outbreaks reported to CDC from 1990-2015. Of 1144 SE outbreaks, 657 (57%) outbreaks were reported with known food items and 487 (43%) were unknown source-based outbreaks. Of 657 known food implicated outbreaks, 273 (24%) are associated with eggs-based products and ranked as first cause of SE outbreaks cumulatively occurred from 1990 to 2015. Meat products are found as second key cause of SE outbreaks 130 (11%) followed by other implicated food items; vegetables 96 (8%), chicken items 95 (8%), dairy products 55 (5%), and bakery items 8 (1%) in the country. **(Table 1)** It was analyzed descriptively by making categories of outbreaks associated food vehicle i.e. simple (single food implicated) and complex (one main high-risk item along with low-risk multiple food items). Of 273 egg-based outbreaks, 93 (8%) were simple egg-based and 180 (16%) complex based outbreaks. Of 130 meat products, 75 (7%) are simple and 55 (5%) were complex category outbreaks. Similarly, of 95 (8%) chicken, 53 (4%) simple and 42 (4%) complex. Of 96 (8%) vegetables outbreaks, 75 (5%) simple and 21 (2%) complex category-based outbreaks. Among 55 (5%) dairy products, 44 (4%) simple and 11 (1%) complex category-based outbreaks. **(Table 1, Figure 6)**

These categories were made based on the disease risk associated with food items reported in previous literature in the country and world. Accordingly, eggs-based dishes were highly associated with SE outbreaks and were given highest rank in the risk order list.

Table 1: Distribution of different food categories implicated in *Salmonella Enteritidis* outbreaks in USA (1990-2015)				
Food Vehicles	**Sub-category Frequency**	**Percent**	**Total Frequency**	**Percent**
Egg-based dishes			273	23.86
Simple Egg-based dishes	93	8.13		
Complex Egg-based dishes	180	15.73		
Chicken Items			95	8.30
Simple Chicken Items	53	4.63		
Complex Chicken Items	42	3.67		
Meat Products			130	11.36
Simple Meat Products	75	6.56		
Complex Meat Products	55	4.81		
Vegetables			96	8.39
Simple Vegetables	75	6.56		
Complex Vegetables	21	1.84		
Dairy Products			55	4.81
Simple Dairy Products	44	3.85		
Complex Dairy Products	11	0.96		
Bakery Items				
Simple Bakery Items Only	8	0.70	8	0.70
Unknown	487	42.57	487	42.57
*Foods are divided into two categories, simple and complex, where simple means that it was only major ingredient and complex means that there were some other ingredients along with this major ingredient.				

Chicken was ranked as a second major risk factor followed by meat, vegetables, dairy products and bakery items. There is a possibility that this classification may overestimate the high order food items association with SE outbreaks such as eggs and chicken. For that reason, we further analyzed all possible combinations of different food items to see which foods are more likely to be implicated together in SE outbreaks **(Table 3).** In case of eggs-based complex

outbreaks (180), interestingly, the dairy items and eggs together were highly reported 21% (37/180) followed by vegetables 13% (24/180), meat 10% (18/180) and bakery 8% (15/180). It gives an idea of how much other food items are involved and implicated along with eggs in causing SE outbreaks reported to CDC from 1990 to 2015. There are chances of underestimation and underreporting of food items other than eggs implicated in outbreaks. This underestimation might be a reason of lack of regulations and preventive measures taken against these food items to reduce SE occurrence in the country. Similarly, in case of chicken, major food items implicated with chicken items in SE outbreaks are vegetables 55% (23/42) and meat 14% (6/42). Next, of 55 meat based complex outbreaks, vegetables are the main food item reported jointly with meat products in 27 (49%) outbreaks followed by dairy and meat items together in 15 (27%) outbreaks. Furthermore, of 21 vegetables based complex category outbreaks, both dairy and bakery items were reported separately with vegetables in 8 (38%) SE outbreaks **(Table A).** Overall, vegetables are the most frequent food items which are found jointly with implicated food items such as eggs, chicken and meat items in SE outbreaks from 1990 to 2015.

This shows that vegetables items and meat products are also playing key role in causing of SE outbreaks in the country which has not been addressed properly in past years. However, few regulations exist to reduce SE outbreaks caused by these food items.

4.2 Temporal Changes in Food Categories Associated with SE Outbreaks

Next, we explored the overall temporal changes among these food vehicles associated SE outbreaks in USA. A plot was drawn between the numbers of outbreaks per food vehicle versus years starting from 1990 to 2015 **(Figure 1).**

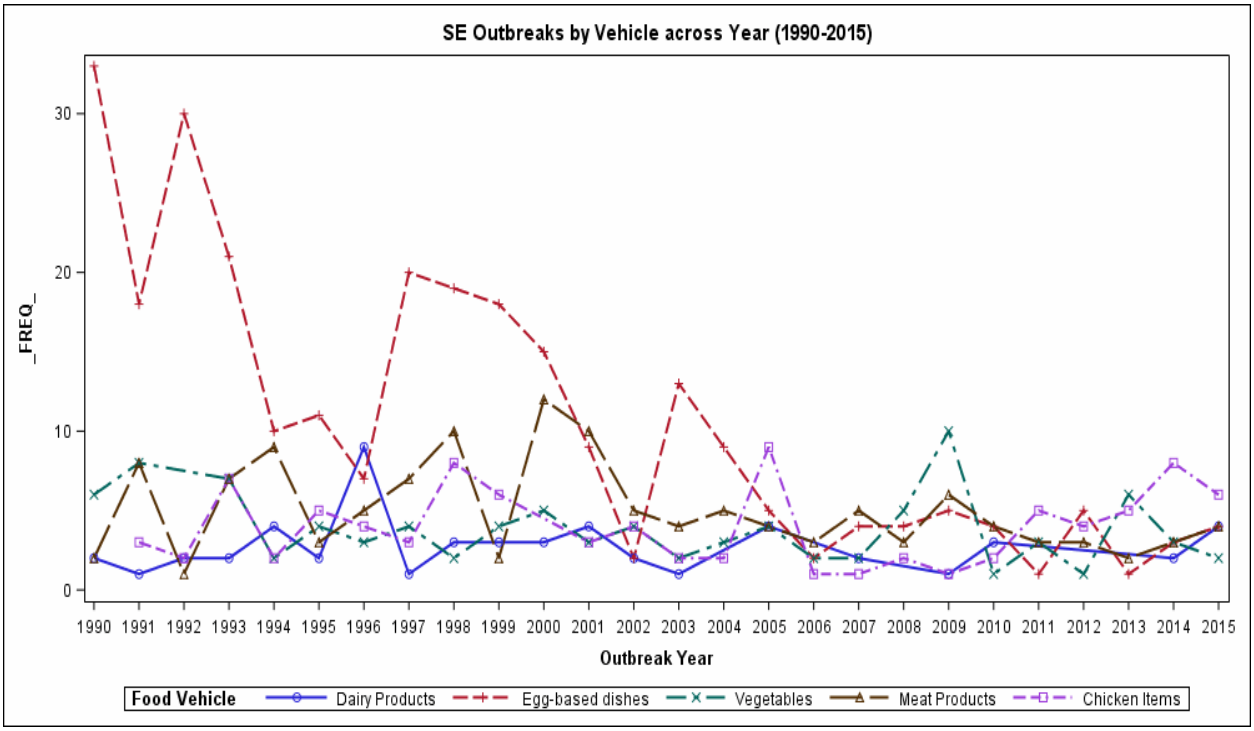


Figure 1: Description of the trends of *Salmonella Enteritidis* outbreaks associated with different foods sources from 1990-2015 in the USA.

We only kept major known sources in this plot, removed the bakery and unknown items **(Figure 1).** Next, we also divided the study period (26 years) into groups of 5 years except the last group which is based on 6 years period **(Figure 7).** In both figures, eggs associated outbreaks were dominantly higher than other known food sources until 2005 and afterward, when other food sources i.e. meat, chicken, vegetables, caused more outbreaks or at similar rate as eggs. Importantly, the number of chicken associated outbreaks has been increasing from 2009 to 2015. Moreover, the occurrence of SE outbreaks by food sources other than eggs remained a consistent source of outbreaks since 1990s. It shows that we need to pay attention to these other food sources instead of only focusing on eggs alone to control the diseases occurrence at single and multistate level outbreaks in the country.

4.3 Geographic distribution of SE outbreaks in the USA

In the current study, we observed the distribution of SE outbreaks occurred in District of Columbia (Washington D.C.) and other 48 states of the USA.

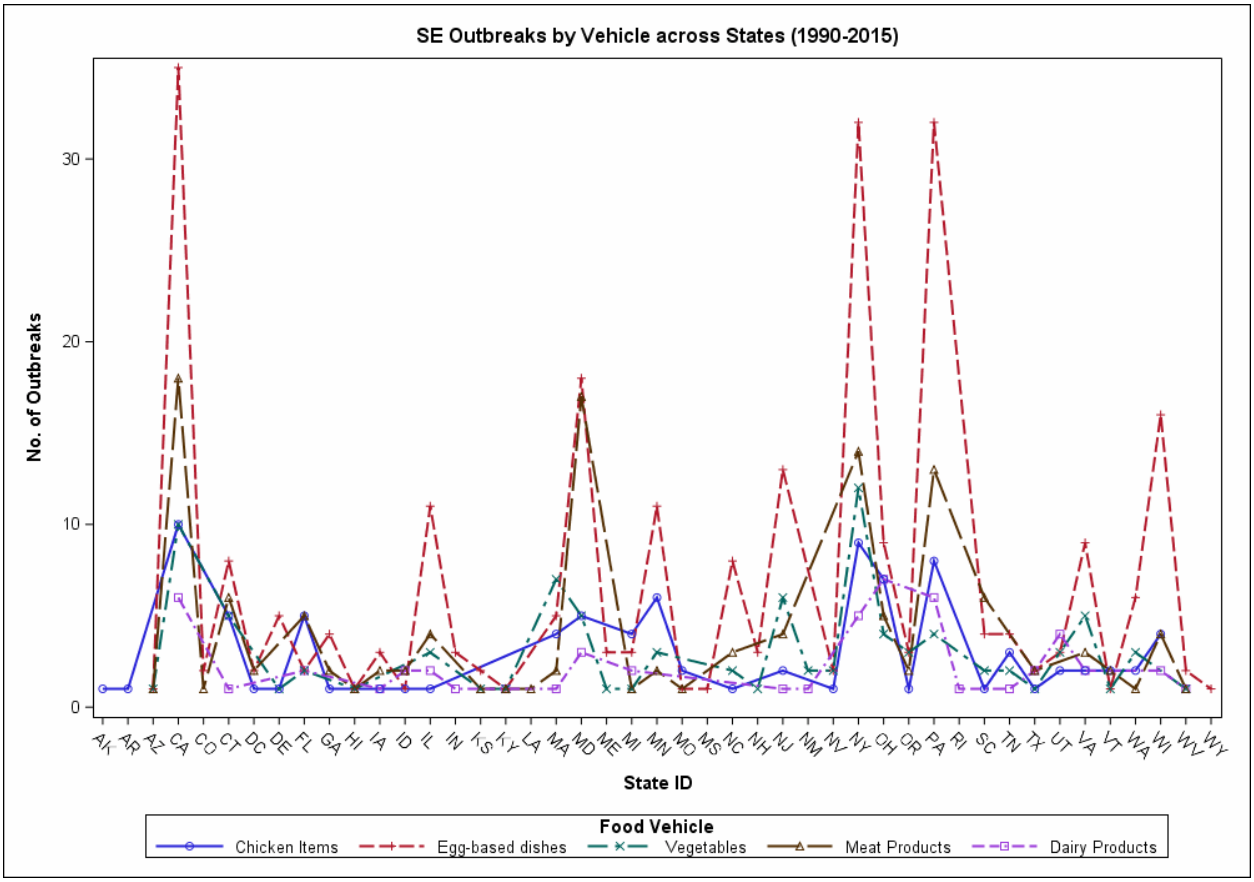


Figure 2: Distribution of *Salmonella Enteritidis* outbreaks based on main known food sources, excluding bakery items across the USA from 1990-2015.

In figure 2, the number of different food categories associated SE outbreaks were plotted across states in the country. It was found that overall, highest number of outbreaks were reported by CA (130) followed by NY (127), PA (98), OH (70), MD (69), MA (52), IL (49) and NJ (47) to CDC from 1990-2015. However, during this study period, eggs-based dishes were the dominant source of associated SE outbreaks throughout the country. However, there was some variation in the second most attributed food source across states. Meat products were second

major cause in high ranked states i.e. CA, NY, CT, MD and PA while vegetables in (VA, NJ, WA) and chicken items in (MN, WI) were second major source of SE outbreaks after eggs **(Figure 2).** It shows a diversity in food items associated with outbreaks across the country. Each state may declare regulations against specific food items which are more associated with the disease occurrence there.

Next, we explored the distribution of outbreaks associated with different food sources across different census regions. Of 1144 SE outbreaks, 402 (35%) outbreaks occurred in Northeast region followed by South 253 (22%), West 250 (22%), and Midwest regions 239 (21%). As this distribution is based on aggregate count of outbreaks from 1990 to 2015 so, we further analyzed the trend of outbreaks that occurred in the census regions on a per year basis since 1990. We found **(Figure 3)** that the highest number of outbreaks occurred in Northeast region in early 1990's but afterwards, disease had spread and become endemic through the country. It is clear from the figure that more outbreaks are currently being reported from South, West and Midwest regions than Northeast region.

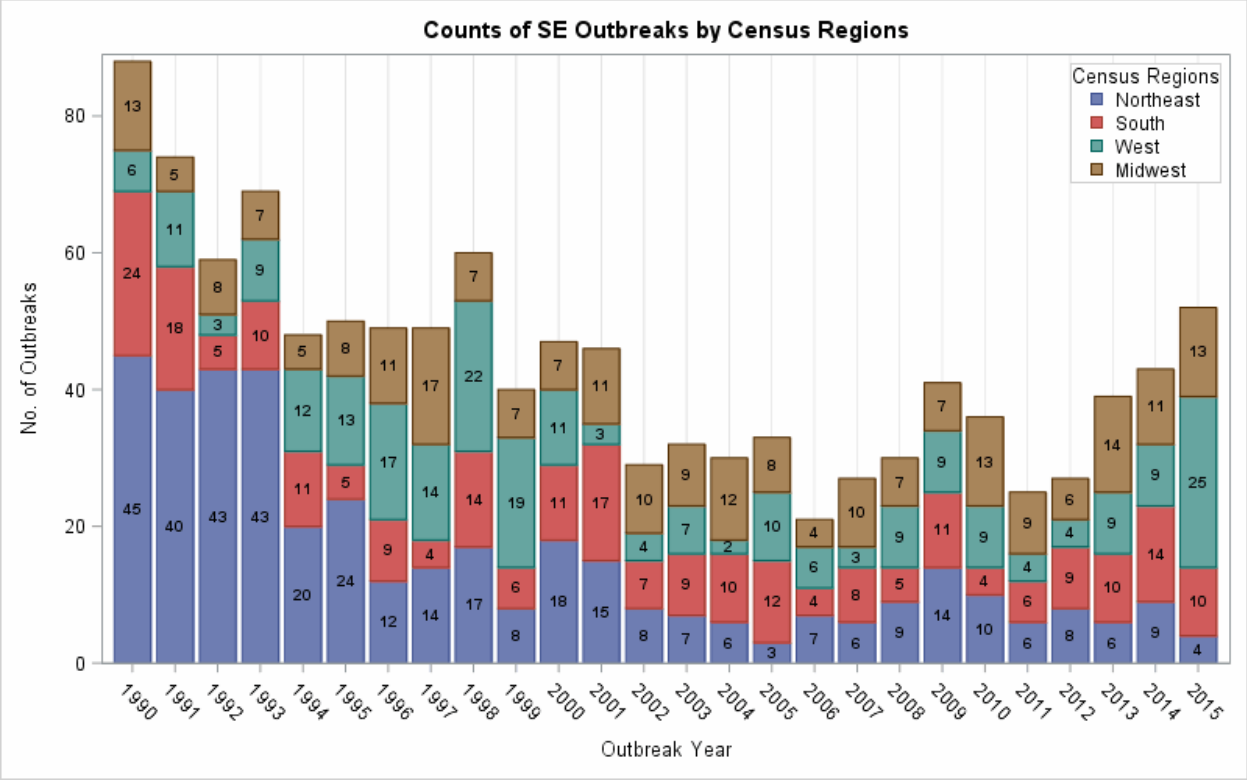


Figure 3: Description of *Salmonella Enteritidis* outbreaks occurred in census regions of USA from 1990-2015.

In a different analysis, we plotted a graph between census region outbreaks and five distinct groups of years. It was observed that Northeast region reported higher number of outbreaks from 1990-1994 but interestingly in the next five years, from 1995-1999, West region reported higher number of outbreaks. This might be due to an emergence of PT4 in West region that caused too many outbreaks simultaneously. In addition, from 2010 to 2015, Midwest region showed an increasing trend of outbreaks followed by West, South, Northeast regions. It shows a changing pattern of outbreaks across time and regions **(Figure 8).**

We were interested to see the changes in occurrence of SE outbreaks in census regions based on known and unknown food items. Based on summarized the data, **Figure 4** shows that overall, Northeast region reported high numbers of outbreaks associated with both unknown and

major known food categories. South region reported meat products associated outbreaks as high as eggs-based. However, we saw differences in food items associated at the state level, but in case of census regions, this effect was diminished, and all census regions showed similar trends of food vehicles associated with SE outbreaks.

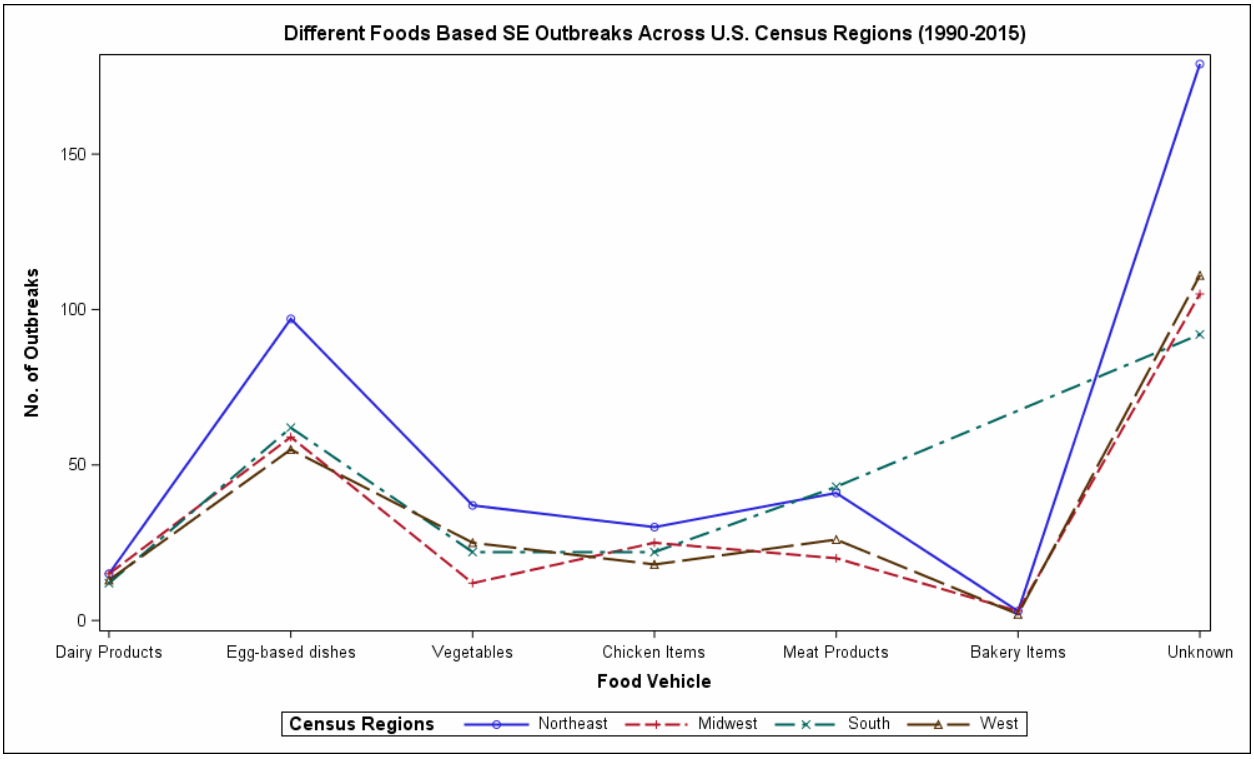


Figure 4: Distribution of number of *Salmonella Enteritidis* outbreaks based on different foods across four census regions of the country.

We further studied the distribution of different foods-based outbreaks in HHS regions of the country because most of the health policies are designed based on the HHS statistics. This regional distribution has much less variability than census regions. It was found that HHS region 3 reported the highest number of outbreaks 225 (20%), followed by HHS region 5, 210 (18%), HHS region 2, 174 (15%), HHS region 9, 155 (14%), HHS region 1, 130 (11%), and HHS region 4, 109 (10%). Figure 9 provides additional information regarding SE outbreaks occurred yearly

in HHS regions. Next, different patterns of HHS regional outbreaks based on food items can be observed in figure 5, where almost all regions showed high numbers of outbreaks associated with eggs-based dishes. Particularly for eggs associated outbreaks, region 3 had higher number of outbreaks followed by HHS regions 5, 2 and 9. In case of meat associated outbreaks, region 3 has significant higher outbreaks followed by HHS regions: 9, 2, 3,4 and 5. For chicken, HHS regions 7 and 3 reported higher outbreaks than others. Similarly, HHS regions 2, 3, 1, 9, 5, and 4 reported more vegetables-based outbreaks **(Figure 5).**

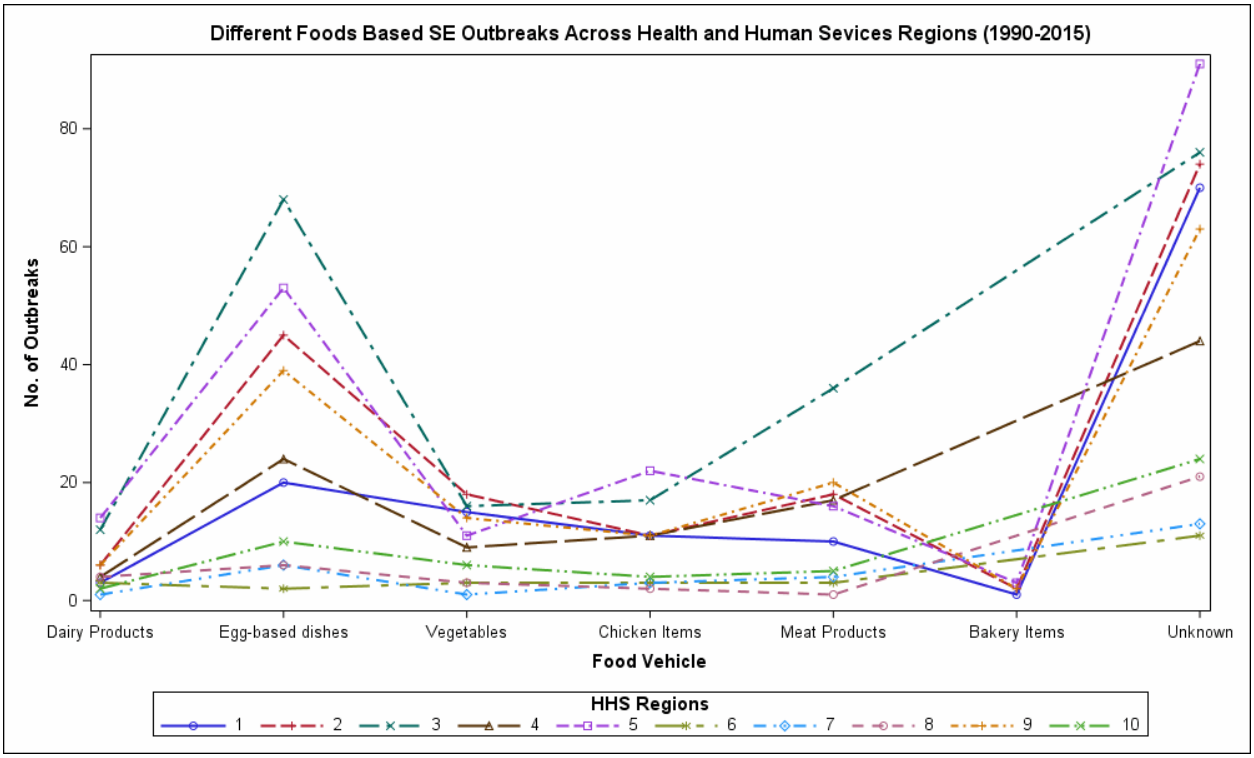


Figure 5: Distribution of *Salmonella Enteritidis* outbreaks across ten HHS regions of the USA from 1990-2015.

4.4 Seasonality of SE Outbreaks

Current study also analyzed the seasonal effect on occurrence of SE outbreaks in the USA. It was found that the highest number of outbreaks 437 (38%) in Summer followed by Fall

282 (25%), Spring 260 (23%), and Winter 165 (14%). We created a plot between season and census regions to show the pattern of regional outbreaks across seasons. We found that almost all four census regions showed a similar pattern of outbreaks over the four seasons **(Figure 10).** Similarly, HHS regions showed a similar trend of season-based outbreaks like census regions where higher number of outbreaks occurred in Summer followed by Fall, Spring and Winter. However, HHS region 2 reported high number of outbreaks in both seasons Summer and Fall at same rate **(Figure 11).**

Spatial visualization in Figure 12, clearly shows an endemicity of the disease in the country. It was seen that in distinct groups of years, there was a switching pattern of the disease outbreaks and associated case rate, calculated per state population, in the country. It might be due to delayed or less interest of state food authorities to develop new preventive measures or to implement policies swiftly against SE disease outbreaks. Initially, SE outbreaks were reported frequently in Northeast region in early 1990's then the trend of high outbreaks moved to West region, and now outbreaks are happening more in South and Midwest. It seems that delay in response against SE infection or any other food borne disease can spread throughout the country after some time.

4.5 Location of SE Outbreaks

Furthermore, we also explored the trend of foods-based outbreaks and the various locations where these outbreaks occurred. It was found that of 1144 outbreaks, there were 1038 outbreaks with known locations and 106 outbreaks were without unknown locations. Overall, the highest number of outbreaks 645 (56%) occurred at restaurants or hotels followed by private home residences 190 (17%), institutions 84 (7%) and nursing facilities or hospitals 65 (6%). It was found that most of SE outbreaks occurred at restaurant and private residence in case of all

food categories. This is consistent with previous findings, for example, a recent study showed that restaurants with sit-down places were reported highly associated with food borne outbreaks 373 (48%) in 2015. It shows that restaurants are still the common places associated with food borne outbreaks in the country (12).

4.6 Statistical Analyses

Negative binomial and Poisson regression models were applied to assess the impendent and joint association of food vehicles in SE outbreaks. We analyzed counts of SE outbreaks per year, classified by different food categories from 1990 to 2015. In the final model, food categories were added as categorical variables and year as a continuous variable to estimate the expected count of SE outbreaks associated with each food category.

Table 2: Food Vehicles Implicated in *Salmonella Enteritidis* Outbreaks in USA

Food Vehicles	Estimates	Lower 95% CI	Upper 95% CI	P-value
Bakery	1.77	0.81	3.85	0.1493
Chicken	6.02	4.42	8.20	<.0001
Dairy	3.85	2.72	5.44	<.0001
Meat	7.34	5.55	9.71	<.0001
Vegetables	5.71	4.22	7.72	<.0001
Eggs' Dishes	14.40	11.38	18.23	<.0001

Estimate of expected count of SE of outbreaks by food vehicles based on Negative binomial regression with food vehicle categories from 1990 to 2015. Eggs-based dishes were considered a referent category in the model.

The estimated count of SE outbreaks associated with eggs-based dishes per year is 14.4 outbreaks in the USA when all vehicle categories are held constant. After eggs, the expected numbers of SE outbreaks associated with meat products per year in the USA will be around 7.3 (Table 2). Similarly, expected count of SE outbreaks associated with meat vegetables per year in the US will be around 5.7 and expected count of SE outbreaks associated with chicken-based products will be 6.0 for a year in the USA. However, all food vehicles were found statistically

significant source to cause considerable number of SE outbreaks in the country instead of only bakery items (**Table 2, Table 5**).

In addition, we calculated relative occurrence (rate ratio) of different food categories compared to eggs-based outbreaks based on the same above mentioned Negative binomial model.

Table 3: Relative Occurrence of Food Vehicles Compared to Eggs-based *Salmonella Enteritidis* Outbreaks

Food Vehicles	Estimates (Rate Ratio)	Lower 95% CI	Upper 95% CI	P-value
Bakery vs. Eggs	0.12	0.06	0.27	<.0001
Chicken vs. Eggs	0.42	0.30	0.58	<.0001
Dairy vs. Eggs	0.27	0.18	0.39	<.0001
Meat vs. Eggs	0.51	0.38	0.69	<.0001
Vegetable vs. Eggs	0.40	0.29	0.55	<.0001

This table shows that overall, occurrence of meat products associated outbreaks is higher than chicken associated outbreaks compared to eggs. Similarly, vegetables and chicken associated outbreaks showed almost equal estimates when both were compare individually with eggs-based outbreaks. It clearly shows that vegetables and meat products are critical source of causing SE outbreaks in the country similar as eggs and chicken. In addition, dairy associated products showed significant estimate compared to eggs-based outbreaks. Overall, these finding prove our hypothesis that other food sources are also playing crucial role in occurrence of SE disease along with contaminated eggs and chicken products in the country.

CHAPTER 5: DISCUSSION

5.1 Interpretations

In this study, all SE outbreaks, from 1990 to 2015, with available information on CDC website were included to analyze the distribution and relative occurrence of different food vehicles associated with SE outbreaks in the USA. By considering different dominant food vehicles in this study, it gave us an idea that how could other foods be a potential source of spreading the disease in the country besides eggs-associated outbreaks.

In the analysis, it was found that, overall, eggs-based dishes 273 (24%) were dominantly reported to be associated with SE outbreaks in the country. It was seen that highest number of eggs-based outbreaks were reported in following years, 1990-1993 and 1997 (Figure 1), and after 2005, a significant decline was observed in eggs-based outbreaks in the country. It clearly shows the success of different interventions introduced by USDA and FDA to control eggs-associated outbreaks in the country. These findings are consistent with previously published studies on eggs-based outbreaks in the USA (4,21). However, it is an important to know that in the current study, these food categories were made based on the level of disease risk associated with food items reported previously in the country and the world. In the past, studies didn't address all food vehicles associated with SE outbreaks together in one study. To address this, we devised a strategy of making classification of different food items. According to that, eggs-based dishes are highly associated with SE outbreaks followed by chicken, meat, vegetables, dairy products and bakery items. It means eggs-based dishes, complex category, can contain any other food item along with eggs implicated in outbreaks during the study period. Similarly, chicken complex category can contain rest of food items i.e. meat, vegetables, dairy and bakery, but not eggs products. Therefore, this classification may overestimate the risk and numbers of outbreaks

associated with higher order food items i.e. eggs, chicken relative to meat, vegetables and dairy. It was found that even after putting meat and vegetables on lower order than eggs and chicken, results showed that meat and vegetable products are more implicated in SE outbreaks than chicken items reported to CDC from 1990 to 2015 **(Table1)**. Though, food classification added bias to eggs-based and chicken food categories, but it clearly showed the importance of other food items with less bias; meat, vegetables and dairy products in causing SE outbreaks those were not well-addressed in past studies.

Healthy people goal was made to reduce the Salmonella infections up to 11.4 until 2020 when baseline infection rate was 15.0 per 100,000 population in 2006-2008 (47). It was found that average isolation rate of Salmonella serotypes was 14 cases per 100,000 population from 2004 to 2011 which was higher than healthy people goal 2010 (6.8 per 100,000) (48). Current study also shows increasing and consistent trend of SE outbreaks and cases in the country.

About negative binomial model, it was found that addition of other categorical variables such as season, census regions, along with food vehicles in model gave an outcome with underestimated coefficients for each food category associated SE outbreaks. For the current study, we calculated expected count of outbreaks, and relative occurrence of different food vehicles compared to eggs-based SE outbreaks by using negative binomial model. However, the variable, year, was added in our model as a continuous variable to keep the number of outbreaks per year constant throughout the study period.

5.2 Strengths

Unlike the past studies, it's the first study which has encompassed all possible food vehicles associated with SE outbreaks in the US since 1990 to 2015. This study has addressed the importance of food vehicles which are playing a critical role in endemicity and persistent

occurrence of SE outbreaks in the country. We observed changes in occurrence of SE outbreaks at state level, census and HHS regions, which showed a variation among these regional classifications and state level reported outbreaks. It suggests that policies should be designed individually based on the local state. Moreover, it adds a concern that how preventive policies can be differed based on chosen classification of regions in the country.

5.3 Limitations

Dataset has following few limitations; first, all outbreaks are not reported and investigated. Outbreaks data alone is not good marker to study endemicity and prevalence of the disease. However, outbreaks data can provide a considerable overview of the disease occurrence and severity in the country which importance can't be underestimated especially when there is not enough information available about prevalence of sporadic cases associated with SE disease. Currently, FoodNet collects an incidence data of different reportable diseases from 10 different regional sites of the country by active surveillance which gives a good estimate of current disease status but can't represent the actual incidence of the disease in other states and country.

Second, almost half of outbreaks were reported with unknown food source. It might reduce estimates of other reported food vehicles. However, we believe that unknown food category would represent kind of same proportion of the reported foods.

Third, this study analysis based on the date when we accessed the data from CDC website. There is possibility of modification or deletion of outbreaks and illnesses reported to CDC. In this way, findings may vary from already reported in previous studies. Additionally, all cases reported with outbreaks cannot give precise estimates of actual cases in the country.

In addition, we also excluded multistate outbreaks from all of study analyses thinking that it might influence overall estimates. Additionally, for the sake of consistency in all analyses, multistate outbreaks were excluded otherwise, it was unable to see changes across states, census regions and HHS regions. However, recent report showed that 30 multistate food borne outbreaks were reported in 2015 and of 30, 17 outbreaks were caused by Salmonella serotypes, and specifically 2 of 17 outbreaks were caused by SE (10).

CHAPTER 6: CONCLUSION

In this study, it was aimed to investigate different food vehicles associated with SE outbreaks beside eggs in the USA. By exploring the distribution and trend of these food associated outbreaks across seasons, census regions, HHS regions, and different time points, we discovered significant details about foods implicated in SE outbreaks from 1990 to 2015 in the country. Study results showed that food vehicles besides eggs: meat, chicken, vegetables and dairy products share significant role in causing and spreading SE disease outbreaks all over the country. Even though, eggs and chicken were put on higher order risk food categories, there were still high number of SE outbreaks associated with meat and vegetables products almost more than chicken items implicated outbreaks. In addition, there was a variation noticed in an order of foods implicated in SE outbreaks at state level which suggests designing state specific preventive measures and regulations. Similarly, study analyses show varied distribution of food vehicles among different census regions and HHS regions. For spatial analysis, we were not able to run geographical weighted regression because the number of outbreaks per state were less than 20. However, it would be worth knowing to design this kind of study at county level in high reporting states to further specify the hotspots of the disease within a particular state. Analysis of different locations implicated in outbreaks showed that private residence was second common place to be associated with SE outbreaks after restaurant. It suggests educating people about the storage, cooking and consumption of foods associated with SE disease.

This study findings suggest that there is dire need to make policies for shipping, selling and serving of these food items particularly meat, vegetables and chicken items at restaurants to stop consistent occurrence of SE outbreaks in the country.

APPENDIX

Table 4: Description of all possible combinations of different foods implicated in *Salmonella Enteritidis* outbreaks in USA (1990-2015).				
Vehicle	**Frequency**	**Percent**	**Cumulative Frequency**	**Cumulative Percent**
Bakery items	8	0.70	8	0.70
Chicken	58	5.07	66	5.77
Chicken Baker	2	0.17	68	5.94
Chicken Dairy	1	0.09	69	6.03
Chicken Meat	6	0.52	75	6.56
Chicken Meat Vege*	1	0.09	76	6.64
Chicken Mix**	4	0.35	80	6.99
Chicken Vegetables	23	2.01	103	9.00
Dairy	44	3.85	147	12.85
Dairy Bakery	8	0.70	155	13.55
Dairy Bakery Meat	3	0.26	158	13.81
Eggs	93	8.13	251	21.94
Eggs Bakery	15	1.31	266	23.25
Eggs Chicken Mix	1	0.09	267	23.34
Eggs Chicken	5	0.44	272	23.78
Eggs Dairy	37	3.23	309	27.01
Eggs Dairy Bakery	1	0.09	310	27.10
Eggs Meat	18	1.57	328	28.67
Eggs Meat Mix	1	0.09	329	28.76
Eggs Meat Vege	1	0.09	330	28.85
Eggs Mix	59	5.16	389	34.00
Eggs Vege Bakery	1	0.09	390	34.09
Eggs Vege Dairy	17	1.49	407	35.58
Eggs Vegetables	24	2.10	431	37.67
Meat	75	6.56	506	44.23
Meat Product	7	0.61	513	44.84
Meat Bakery	1	0.09	514	44.93

Table 4 (cont'd)				
Meat Dairy	15	1.31	529	46.24
Meat Mix	5	0.44	534	46.68
Meat Vegetables	27	2.36	561	49.04
Unknown	487	42.57	1048	91.61
Vegetables	75	6.56	1123	98.16
Vegetables Bakery	8	0.70	1131	98.86
Vegetables Dairy	8	0.70	1139	99.56
Vegetables Mix	1	0.09	1140	99.65
Vegetables Mix	4	0.35	1144	100.00

*Vege: Vegetables

**Mix: it means that the implicated food in outbreaks had other ingredients along with mentioned ones.

Table 5: Analysis of Maximum Likelihood Parameter Estimates								
Parameter		**DF**	**Estimate**	**Standard Error**	**Wald 95% Confidence Limits**		**Wald Chi-Square**	**Prob**
Intercept		1	2.67	0.12	2.43	2.90	500.60	<.0001
yearnew		1	-0.03	0.01	-0.04	-0.02	23.74	<.0001
Vehicle	Bakery Items	1	-2.10	0.40	-2.88	-1.32	27.72	<.0001
Vehicle	Chicken Items	1	-0.87	0.17	-1.20	-0.55	27.70	<.0001
Vehicle	Dairy Products	1	-1.32	0.19	-1.69	-0.95	48.25	<.0001
Vehicle	Meat Products	1	-0.67	0.15	-0.98	-0.37	19.06	<.0001
Vehicle	Unknown	1	0.66	0.14	0.39	0.92	23.60	<.0001
Vehicle	Vegetables	1	-0.93	0.16	-1.25	-0.60	31.84	<.0001
Dispersion		1	0.16	0.03	0.10	0.24		

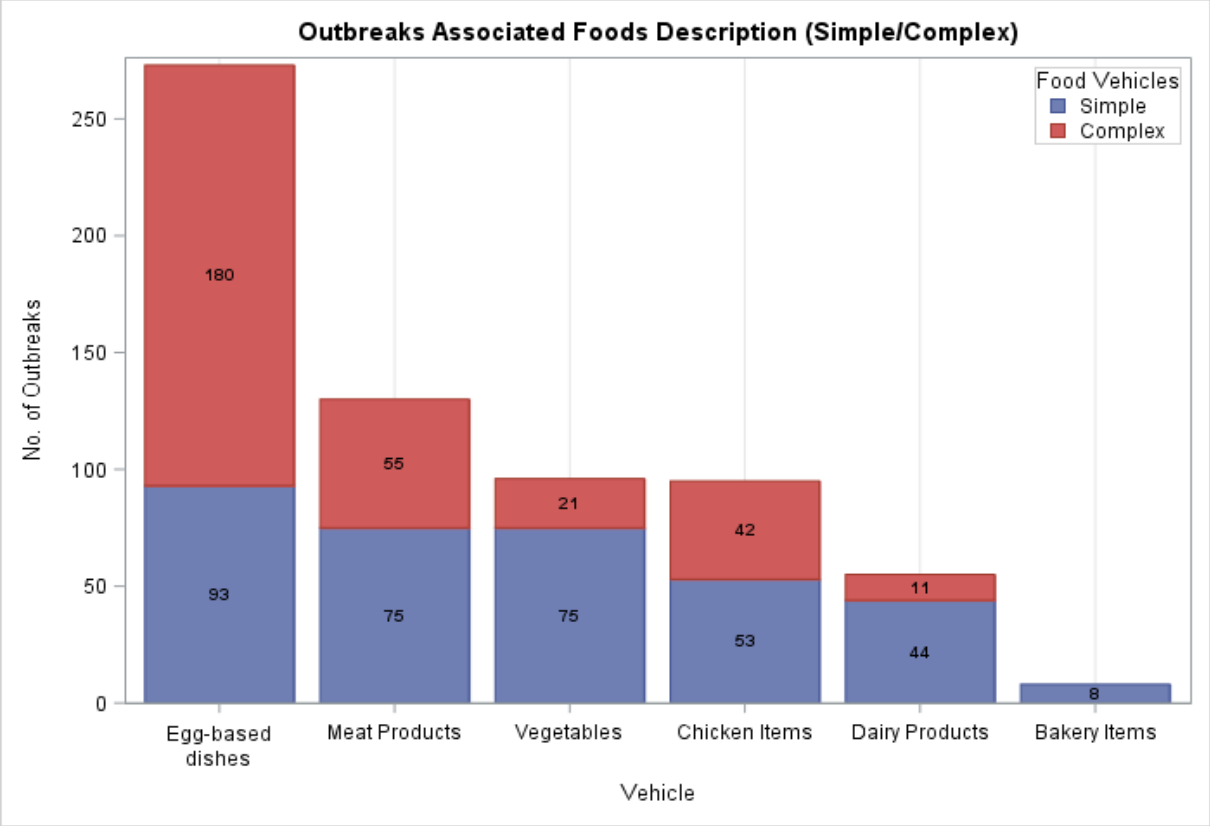


Figure 6: Distribution of different food categories implicated in *Salmonella Enteritidis* outbreaks in USA (1990-2015)

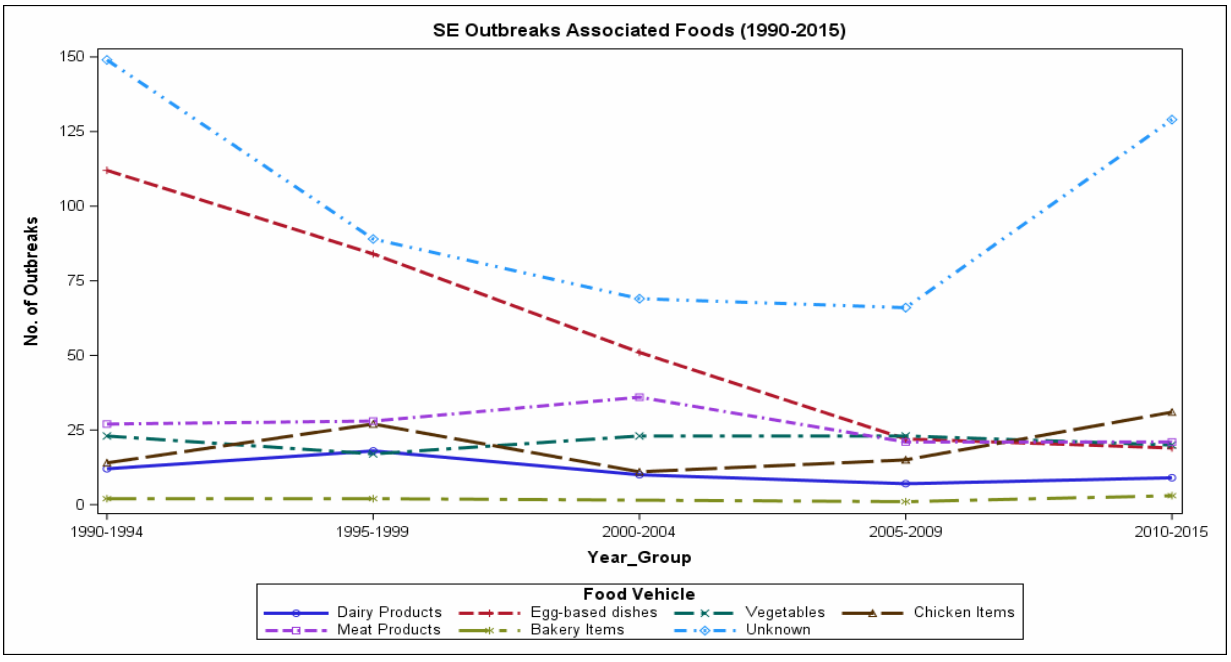


Figure 7: Distribution of foods associated with *Salmonella Enteritidis* outbreaks across five distinct groups of years in USA (1990-2015)

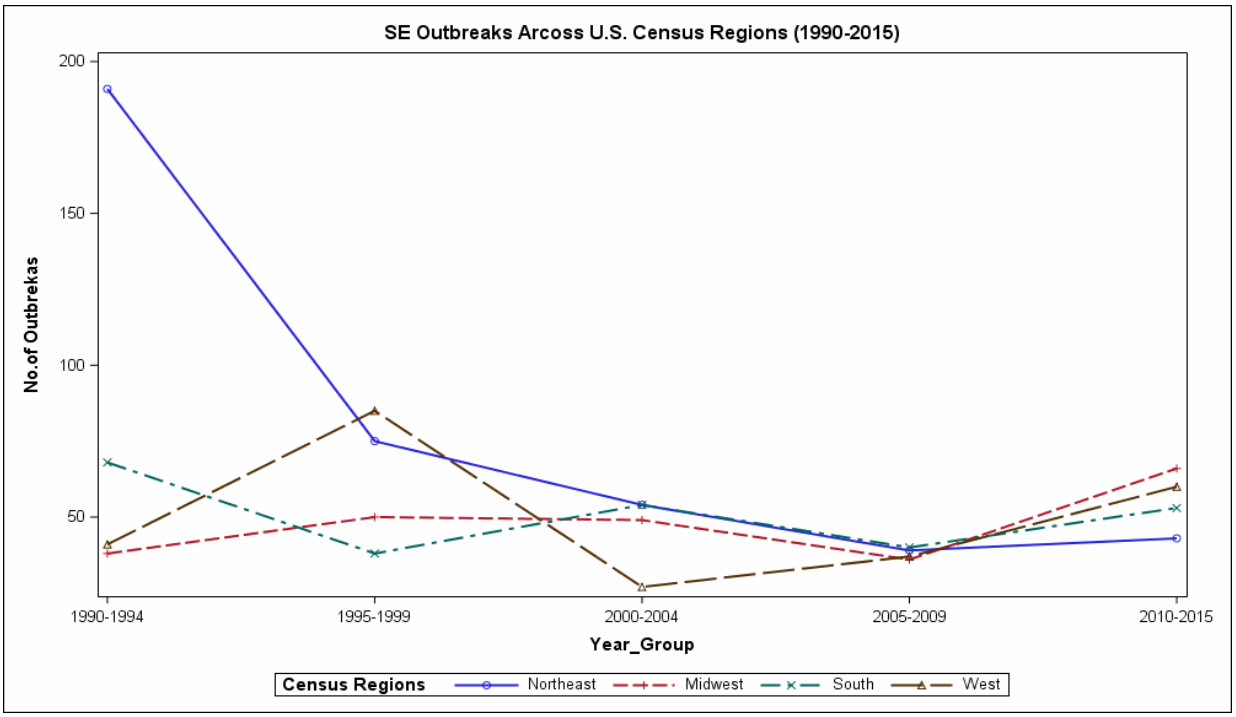


Figure 8: Description of *Salmonella Enteritidis* outbreaks in four census regions of USA: Northeast, Midwest, South, and West.

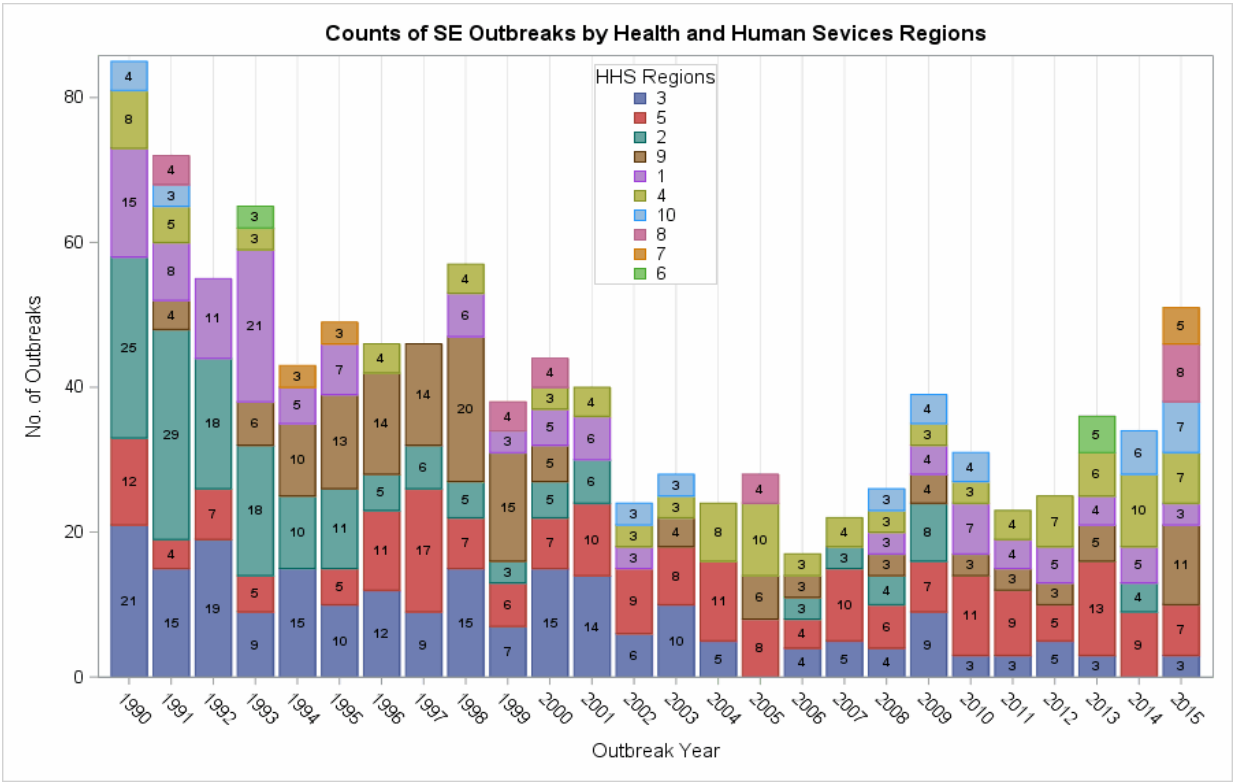


Figure 9: Distribution of *Salmonella Enteritidis* outbreaks across HHS regions which had over two outbreaks per year.

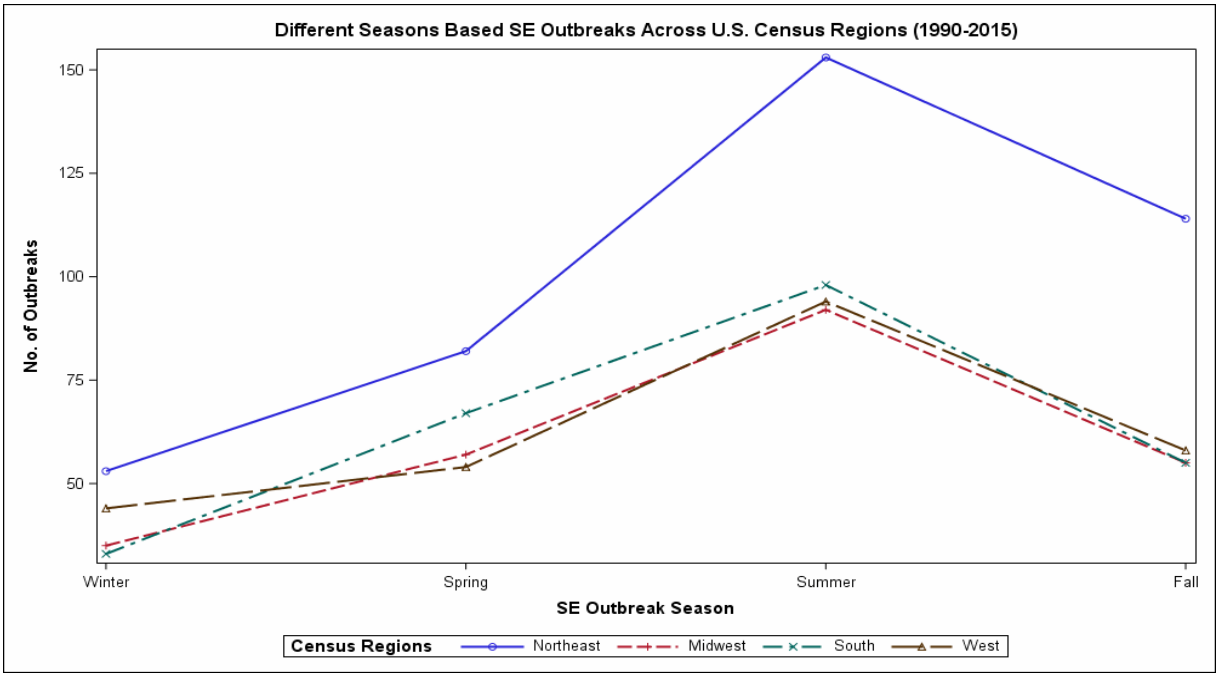


Figure 10: Description of *Salmonella Enteritidis* outbreaks occurring trend in different seasons: Winter, Spring, Summer and Fall across the US census regions from 1990-2015.

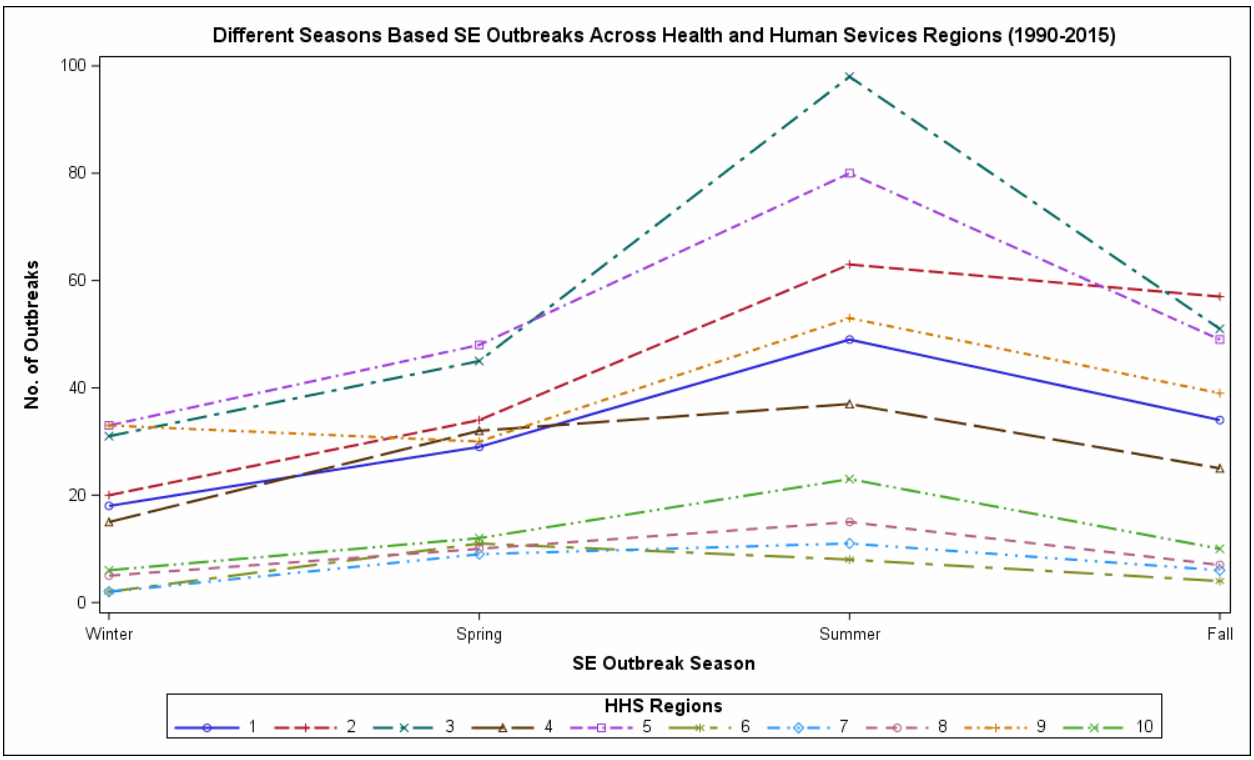


Figure 11: The pattern of different seasons-based *Salmonella Enteritidis* outbreaks in the USA HHS regions from 1990-2015.

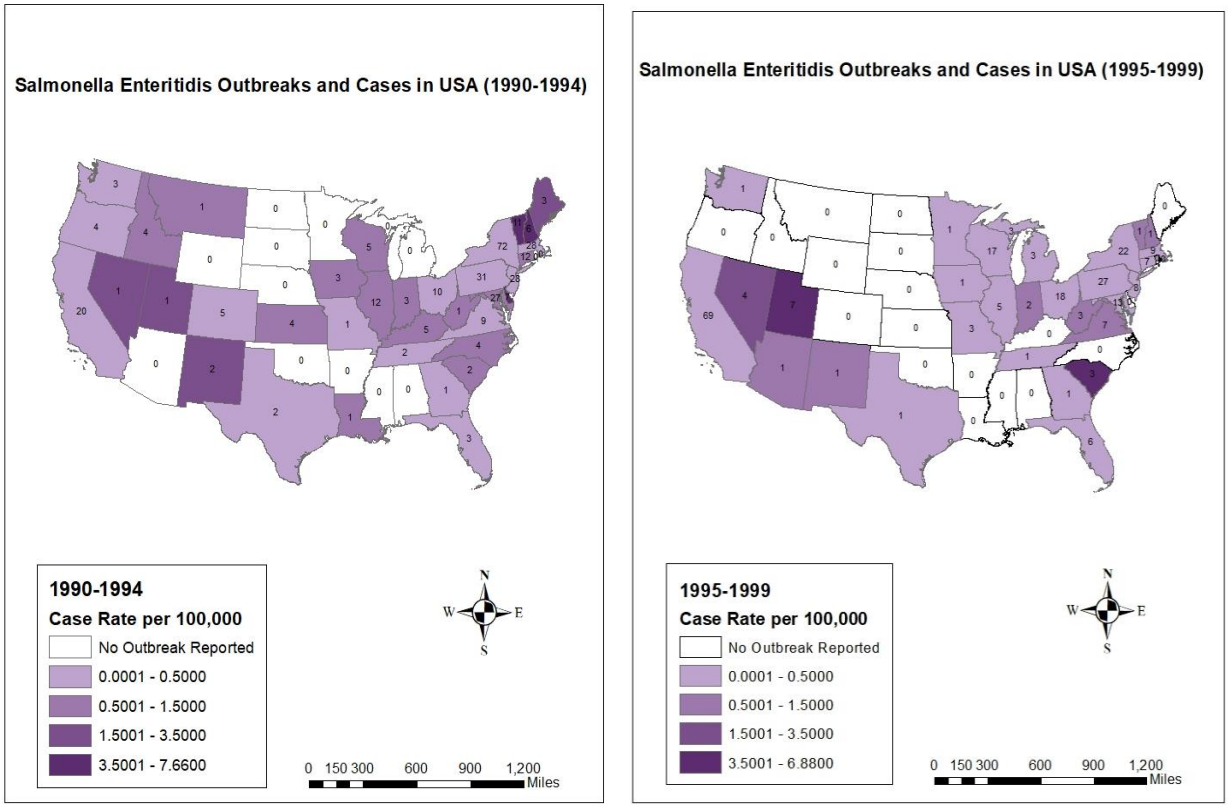
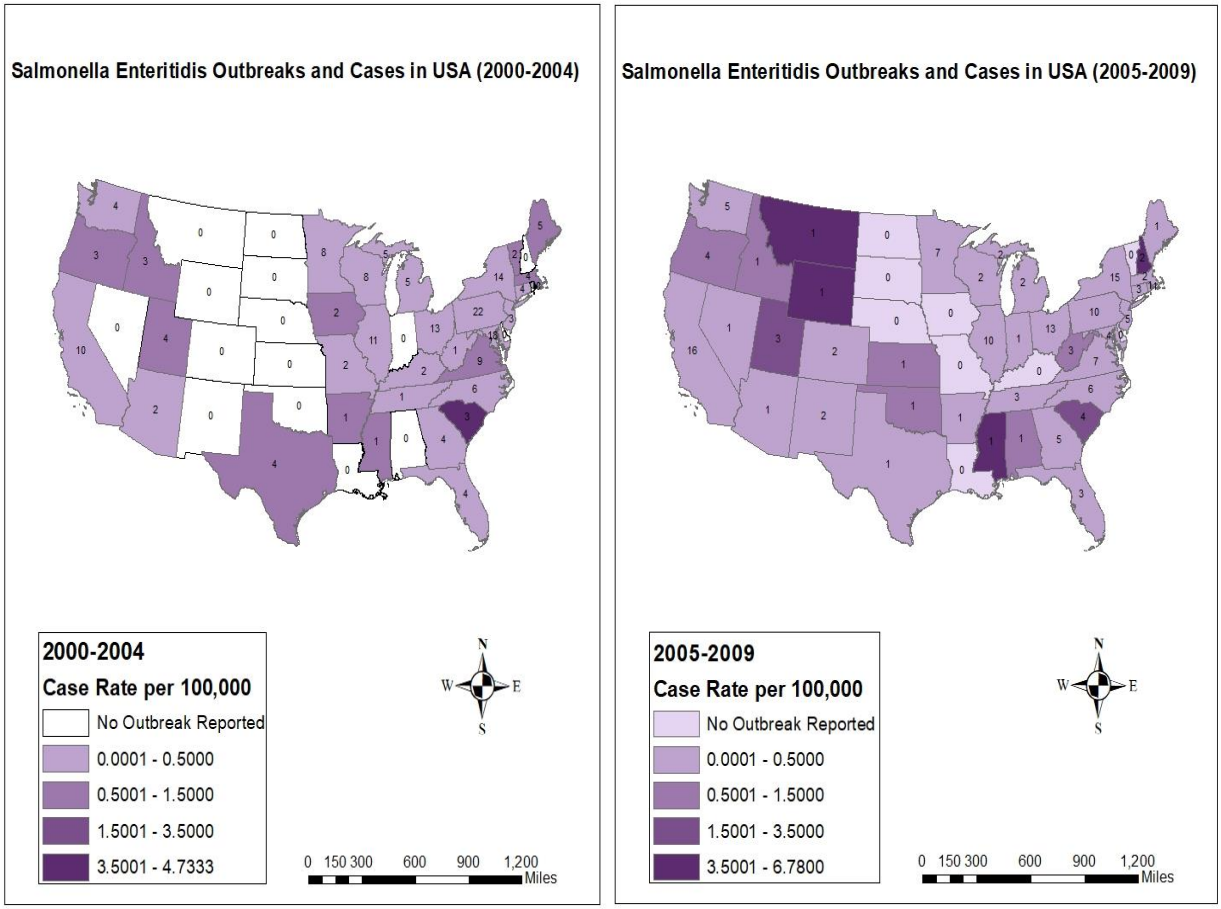


Figure 12: Number of outbreaks and case rate associated with *Salmonella Enteritidis* outbreaks in five distinct group of years in USA

Figure 12 (cont'd)



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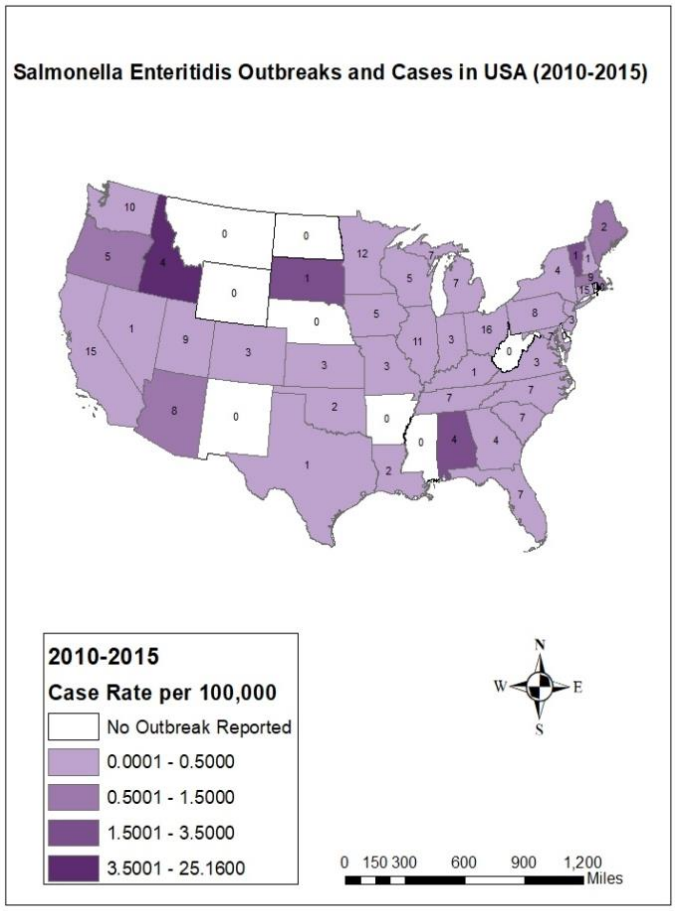

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