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Contaminated Raw Produce as a Major Source of Foodborne Salmonellosis

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2021

Abstract

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by

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MS, University of Maryland University College, 2010

BS, Coppin State University, 2004

Dissertation Submitted in Partial Fulfillment

of the Requirements for the Degree of

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Abstract

Foodborne pathogens continue to threaten the food supply chain. Salmonellosis is a persistent foodborne pathogen responsible for gastroenteritis, leading to severe illness in children below 5 years of age, adults 65 and older, and people with weakened immune systems. Maryland has rising cases of salmonellosis linked to outbreaks due to consumption of raw or fresh fruits and produce. The purpose of this quantitative study was to determine the association between the development of salmonellosis and the consumption of raw (fresh) produce, demographic and geographic characteristics, type of produce, and seasonal variation in Maryland. The study is based on the theory of system thinking and the food supply chain model as the framework. Data were obtained from the Maryland Department of Health and Foodborne Disease Active Surveillance Network for salmonellosis outbreaks between 2010 and 2018. Associations were evaluated using Pearson chi-square analysis and multivariable logistic regression with product terms. Results show summer months and location in the eastern region had the highest associations with occurrence of salmonellosis. Additionally, spinach, watermelon, berries, lettuce, and herbs are significantly associated with salmonellosis outbreaks. With exception of the category non-Hispanic, no other demographic factors showed an association with the development of salmonellosis. The social change implications of my findings include the provision of information to produce farmers, consumers, and local health educators regarding the mechanisms of *Salmonella* transfer and prevention through the entire food supply chain.

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Dedication

To my mother, a single parent who gave me her very best, and my elder brother, Elder Sunday Ikpe who filled the role of a father figure in my life. You both believed in my dreams.

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Chapter 1: Introduction to the Study

Introduction

There is an unprecedented increase in consumer choice of fresh fruits and produce, which constitute menu items in burgers, salads, and sandwiches. The primary risk factor implicated in this food supply chain is *Salmonella enteritidis*, identified to be the leading cause of bacterial foodborne disease resulting in approximately 42,000 cases annually in the United States (Scallan et al., 2011). In the past, salmonellosis outbreaks were said to be zoonotic and always associated with meat products and egg-based dishes (Gould et al., 2013). However, recent studies have linked outbreaks to fresh fruits and produce (Hanning et al., 2009). Irrigation water and static ponds are also potential environmental sources of *Salmonella* contamination of preharvest produce and vegetables that could cause outbreaks (Ijabadeniyi et al., 2011). Emerging evidence suggests that persistent salmonellosis outbreaks in humans from consumption of fresh produce are due to the formation of biofilm on raw vegetables that are resistant to disinfection treatment (Yaron & Römling, 2014).

Results and findings from this study will provide a strong basis for collaboration between Maryland communities and the Food and Drug Administration (FDA) under the Food Safety Modernization Act to ensure the application of microbial water quality standards to the waters used to grow, harvest, and pack fresh produce. Additionally, this study will help educate and encourage Marylanders, both farmers and citizens, regarding the importance of participating in the food safety farm-to-fork program. This study may offer suggestions for the statewide implementation of preharvest practices, including good agricultural practices and postharvest measures such as the Hazard Analysis Critical Control Point (HACCP). “HACCP is a management system in which food safety is addressed through the analysis and control of biological, chemical, and physical hazards from raw material production, procurement and handling, to manufacturing, distribution and consumption of the finished product” (U.S. FDA, 2018)

Understanding the effects of pathogens in consumer food safety—their types, mode of transfer, symptoms, and prevention—continues to be of utmost concern to public health. A typical pathogen is *Salmonella,* which is responsible for several types of gastroenteritis. This inflammation of the stomach and intestine manifests with symptoms such as fever, abdominal pain, diarrhea, nausea, and vomiting and has an incubation period of 12 to 72 hours, lasting for 4 to 7 days (Kovacic et al., 2017).

This chapter contains the background to the study, problem statement, purpose of the study, and research questions. Furthermore, the proposed conceptual framework for this study, nature of the study, definitions of terms, assumptions, scope and delimitations, limitations, significance of the study, and implications for social change are discussed. The chapter ends with a summary and transition to Chapter 2.

Background

Salmonella is high among pathogens responsible for foodborne outbreaks in produce. From 2006 to 2017, *Salmonella* accounted for 53.5% of all foodborne disease outbreaks, 32.7% of which are associated with the consumption of produce (Liu et al., 2018). Of these numbers, 128,000 were hospitalized, and 3,000 died either from one or more of 31 known pathogens or unspecified etiological agents (Scallan et al., 2006). One in six Americans (48 million people) become ill annually from foodborne diseases such as salmonellosis (Scallan et al., 2006). Hruby et al (2018) said soils act as a long-term reservoir for manure-derived *Salmonella.* The soil can release the same pathogens into the environment, which is critical to consider in manure management and pathogen contamination prevention.

One possible source of *Salmonella* is groundwater when affected by improperly composted manure. Polluted groundwater can be transferred to freshwater through flow paths of the soil or surface runoff water (Kovacic et al., 2017). *Salmonella* from the gastrointestinal tract of animal feces adapts to the new microenvironment in surface water, rainwater, and surface runoff characterized by deficient nutrients, ultraviolet sunlight, and high or low pH (Liu et al., 2018). This new microenvironment forms a niche surrounded by other bacterial species and human pathogens like *Salmonella* (Liu et al., 2018).

Salmonella is present in surface water bodies such as rivers, lakes, and ponds, while groundwater is a good microbial quality irrigation source (Liu et al., 2018). A new technique has been introduced by the U.S. FDA under the Produce Safety Rule of January 2013 to prevent *Salmonella* contamination by regulating the spray, wash, and irrigation water that may come in contact with fresh produce.

Persistent salmonellosis outbreaks in humans from consumption of fresh produce is due to the formation of disinfection resistant biofilms and biofilm-like structures on the surface of raw vegetables and fresh produce (Liu et al., 2018; Yaron & Römling, 2014). Environmental transmission and related risk factors point to ruminants and cattle as natural reservoirs that are typically asymptomatic while they shed bacteria into the environment (Hrusa, 2016). Groundwater is seldom contaminated by animal feces or human waste leaking from onsite septic systems (Jacobsen & Bech, 2012).

Occurrence of salmonellosis from consumption of contaminated foods such as meat, milk, eggs, tomatoes, celery, and poultry has been investigated. However, there remains a large number of outbreaks that are of unknown etiology. Fresh produce as a group has not been sufficiently studied as a potential source of foodborne salmonellosis. The gap in literature points to an unconfirmed and unspecific path of *Salmonella* contamination of fresh produce. Areas with high density animal production are considered high risk sources in terms of survival, transport, and contamination of raw produce (Jacobsen & Bech, 2012). This study filled this gap by clarifying the role of consumption of contaminated fresh produce as a source of salmonellosis. This information could provide a strong basis for compliance with microbial water quality standards and the application of best agricultural practices to grow, harvest, and pack fresh produce. It will help educate farmers and citizens address implementation of preharvest practices, including good agricultural practices and postharvest measures such as the HACCP, and encourage their use or application in food safety practices.

Foodborne salmonellosis outbreaks are no longer limited to traditional meat and poultry sources. This proposed study uses data wherein salmonellosis outbreaks are implicated in fresh produce, including fruits and vegetables. Fresh produce is found in many menu items and most, if not all, salad dishes. According to Schleker et al. (2015), salmonellosis is the most frequent foodborne disease worldwide and can be transmitted to humans via animal and plants products. This study did not only shift focus from meat and poultry products to fresh produce as sources of *Salmonella*, but also filled the research gap by examining paths of contamination.

Problem Statement

Although previous researchers investigated incidences of salmonellosis due to consumption of contaminated foods such as meat, milk, eggs, tomatoes, celery, and poultry, there remain a large number of outbreaks that are of unknown etiology. Fresh produce as a group has not been sufficiently studied as a source of foodborne salmonellosis. My study investigated the role of fresh produce in the occurrence of foodborne salmonellosis in the state of Maryland.

The recent outbreak of the strain *Salmonella infantis* in the U.S. infected 92 people (median age of 36) in 29 states between January and September 2018, with 21 hospitalized cases (CDC, 2018). *Salmonella* is known to emerge in strains despite continuous advances in food safety as well as disease surveillance, control, and prevention, and has led to several foodborne outbreaks (Le Hello et al., 2012).

The gap in literature points to an unconfirmed and unspecific path of contamination of fresh produce for *Salmonella*; however, areas with high-density animal production are considered high-risk sources for the survival, transport, and contamination of raw produce (Jacobsen & Bech, 2012). The goal of surveillance can be achieved if geographical distribution of implicated food items and characteristics of pathogens are identified and traced to the source of the outbreak. The *Salmonella* pathogen can survive in both wild and domestic animals as part of the normal gastrointestinal microflora, with animals acting as reservoirs and spill over hosts (Persad & LeJeune, 2018). This resident enteric flora is shed in animal feces, but the exact etiology of these cases is still unknown (Liu et al., 2018; Persad & LeJeune, 2018).

Purpose of the Study

In this quantitative study, I examined the relationships between consumption of raw produce, demographic characteristics of the study population, raw produce consumption and seasonal variation, site of outbreak, and agent (vehicle) of transmission, as well as occurrence of salmonellosis. I identified sites of outbreak of salmonellosis by city, county, and region, as well as type of fresh produce. Therefore, the purpose of this study was to investigate major risk factors in salmonellosis outbreaks in Maryland, methodically evaluate their influence on the consumption of raw produce (predictor variable) and determine the relationship between consumption of produce and occurrence of foodborne salmonellosis (dependent variable).

Outbreaks of salmonellosis traceable to fresh produce consumption in Maryland from 2010 through 2018 and major risk factors associated with these outbreaks was the primary focus of this study. Sites of contamination were addressed using data obtained from the Maryland Health Department; this study did not identify sources by name or locations. Types of contaminated produce associated with salmonellosis outbreaks were identified as berries, cantaloupe, herbs, lettuce, spinach, sprouts, tomatoes, watermelon. The study determined relative percentages of salmonellosis occurrence associated with different types of produce.

Research Questions and Hypotheses

In this study, I addressed relationships between salmonellosis outbreaks due to fresh produce, risk factors involved in this outbreak, and those at high risk of contracting the disease in the state of Maryland. The following are the (RQs) this study answered:

RQ1: Is there an association between the consumption of fresh produce and the occurrence of salmonellosis?

$H_0$1: There is no association between the consumption of fresh produce and the occurrence of salmonellosis.

H_a1: There is an association between the consumption of fresh produce and the occurrence of salmonellosis.

RQ2: Is there an association between demographic characteristics (age, sex, race and ethnicity) of the state of Maryland and the risk of contracting salmonellosis through the consumption of fresh produce?

$H_0$2: There is no association between demographic characteristics of Maryland and the risk of contracting salmonellosis through the consumption of fresh produce.

H_a2: There is an association between demographic characteristics of Maryland and the risk of contracting salmonellosis through the consumption of fresh produce.

RQ3: Is there an association between salmonellosis foodborne outbreaks from fresh produce and seasonal patterns in Maryland?

$H_0$3: There is no association between salmonellosis foodborne outbreaks from fresh produce and seasonal patterns in Maryland.

H_a3: There is an association between salmonellosis foodborne outbreaks from fresh produce and seasonal patterns in Maryland.

RQ4: Is the association between consumption of raw produce and development of salmonellosis modified by geographic location in Maryland?

$H_0$4: The association between consumption of raw produce and development of salmonellosis is not modified by geographic location in Maryland.

H_a4: The association between consumption of raw produce and development of *salmonellosis* is modified by geographic location in Maryland.

RQ5: Is the association between salmonellosis outbreaks and fresh produce consumption modified by the type of produce consumed?

$H_0$5: The association between salmonellosis outbreaks and consumption of fresh produce is not modified by the type of produce consumed.

H_a5: The association between salmonellosis outbreaks and consumption of fresh produce is modified by type of produce consumed.

Theoretical Framework

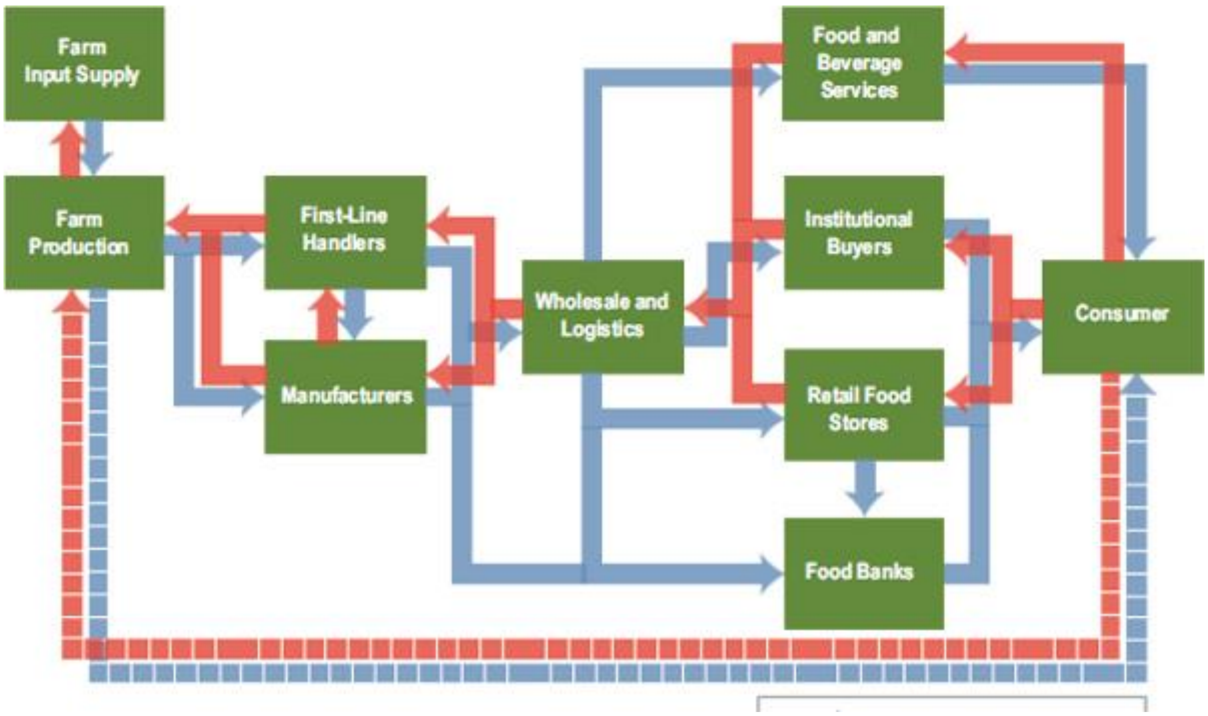
In this study, I used the system theory as the framework for my study. Von Bertalanffy (1968) said system thinking is a complex of interacting components and concepts characteristic of organized wholes as they interact together. According to Kramer and de Smit (1977), system thinking is a way of looking at reality holistically, acknowledging that the environment is an essential part of the system while interacting with that system. Contemporary system thinkers like Wheatley, von Bertalanffy, Wilber, and Meadows all posit a holistic approach to solving complex problems in an organic world with the assumption that everything is interrelated. I used the food supply chain model to identify both interacting and interdependent components of the food supply chain, namely growers (farmers), shippers (transporters), retailers (grocery stores), and the food service industry (restaurants).

The FDA through the Food Safety Modernization Act (FSMA) introduced a new food safety program that involves using the principle of system thinking to connect different stakeholders in the food safety industry and link them to food distribution networks. This is called the New Era of Smarter Food Safety. It encompasses food safety, people-led, FSMA-based, and technology-enabled approach to food safety (Hahn, 2020). It is a holistic approach to solving complex problems of food safety using the principle of system thinking in a way that looks at reality by connecting individual stakeholders to interact with the different components of the food supply system known as farm-to-fork strategy. The New Era of Smarter Food Safety is a platform for FDA food safety experts, consumers, the food industry, technology firms, federal and state regulatory partners, regulatory counterparts in other nations, and academia (Hahn, 2020).

Traceability is paramount during outbreaks of salmonellosis and should be integrated as part of the components of the food safety, system thinking holistic approach. FSMA core elements include tech-enabled traceability, smarter approaches for prevention of food contamination and outbreak responses, new business models and retail modernization, and food safety culture (McMeekin, 2020). Using this legislation, I attempted to address specific agents of transmission of *Salmonella* and demographics of affected populations.

Figure 1

Conceptual Model of a Food Supply Chain



From "*A Framework for Assessing Effects of the Food System* by the Committee on a Framework for Assessing the Health, Environmental, and Social Effects of the Food System; Food and Nutrition Board, Board on Agriculture and Natural Resources, Institute of Medicine, National Research Council, Nesheim, M. C., Oria, M., Yih, P. T., (Eds.), 2015. National Academies Press.

Nature of the Study

This quantitative study involved assessing relationships between the occurrence of salmonellosis and consumption of fresh produce, demographic characteristics of the study population, seasonal variation, sites of occurrence, and type of produce consumed. A cross-sectional quantitative research design was used for this study. This is a type of observational study where both exposure and outcome can be measured at the same time. Secondary data analysis was used for this study. Secondary data are data that have already been collected and analyzed by someone else for another primary purpose (Johnston, 2014). Secondary data analysis involves reanalyzing, interpreting, and reviewing past data for new research inquiries (Oxbridge Research Group, 2020).

Secondary data for this study were obtained from the epidemiological unit of the Maryland Department of Health (MDH) and the Foodborne Diseases Active Surveillance Network (FoodNet). The MDH is one of 10 U.S. state health departments alongside the U.S. Department of Agriculture's Food Safety and Inspection Service (FSIS) in partnership with the U.S. Food and Drug Administration (FDA) which forms FoodNet. FoodNet is an active surveillance network which collaborates with the CDC to surveil gastroenteritis, campylobacter infections, salmonellosis, shigellosis, and other foodborne diseases (CDC, 2020).

The main dependent variable in this study is occurrence of salmonellosis. Independent variables include demographic variables of the study population. This includes age, sex, race, ethnicity, and county of residence. Another independent variable is consumption of fresh produce. Source of infection is another independent variable. Agent of transmission is defined as the type of produce identified as sources of salmonellosis outbreaks. Seasonal variation and site of outbreak are also independent variables which were considered in this study. Specific names of municipalities, institutions, and establishments were expunged from the dataset by the MDH for purposes of confidentiality.

Secondary data which were sourced using a Microsoft Excel spreadsheet which was analyzed with Statistical Package for the Social Sciences (SPSS) version 27. I am also in possession of the code book used for coding of variables in the secondary data. Analysis was based on research questions, hypotheses, and operationalization of study variables. The analysis started with descriptive statistics of variables, which included measures of central tendencies for continuous variables and bar and pie charts for nominal variables.

Definitions

Antimicrobials: A general term that refers to a chemical that inhibits the growth of or kills microscopic organisms.

Biofilms: Complex microbial ecosystems formed by one or more species immersed in an extracellular matrix of different compositions depending on the type of food manufacturing environment and colonizing species (Galié et al., 2018). Fresh produce becomes contaminated when waterborne pathogens adhere to and colonize plant surfaces during pre- or postharvest processes with a thin layer called film that is resistant to disinfectants (Claessen et al., 2014).

Cross Contamination: Cross contamination occurs when *Salmonella* is spread from contaminated food or infected food handler or animal to other foods or objects in the environment. This happens when potentially contaminated raw meats, poultry, seafood, produce, or eggs are not kept separate from each other during preparation or cooking, or when a food handler does not adequately clean utensils, surfaces, equipment, and hands after they have come into contact with these products (FDA, 2014).

Farm-to-fork program: Chains of activities from food production (farm) to consumption (fork), including processing, packaging, distribution, and retail (Verger et al., 2018).

Foodborne: Something that is carried by food, such an illness that is caused by harmful bacteria in or on food (FDA, 2014)

Gastrointestinal: Having to do with the stomach and/or bowel (FDA, 2014).

Hazard Analysis and Critical Control Point (HACCP): "A management system in which food safety is addressed through the analysis and control of biological, chemical, and physical hazards, from raw materials production, procurement, and handling to manufacturing, distribution and consumption of the finished product" (FDA, 2018).

Outbreak: When two or more people become sick due to the same bacterium, virus, or other pathogen, it is called an outbreak (FDA, 2014).

Pathogen: "A life form, such a bacterium or protozoan, that can cause disease. Virus are not life forms, but some cause disease and are among the pathogens" (FDA, 2014, p. 284).

Ready-to-Eat (RTE): Food that is raw or cooked or hot or chilled that is ready for immediate consumption at the point of sale without further treatment (Oranusi & Braide, 2012; Tsang, 2002).

Salmonella: "Salmonella is a motile, non-spore forming, Gram negative, rod-shaped bacterium in the family Enterobacteriaceae and the tribe Salmonellae" (FDA, 2014, p. 9).

Assumptions

Assumptions are essential details or facts in a study upon which the researcher has little control over (Lips-Wiersma & Mills, 2013). For this study, it was assumed that Maryland Department of Health and Foodborne Disease Active Surveillance Network database contained true data for the state of Maryland and due diligence was done during the identification and confirmation of cases of salmonellosis. It was also assumed that information in the database is truthfully stated, and the same standards were used and maintained by compilers for surveillance over the period covered in the database. Lastly, it was assumed that records in the database had not been manipulated and did not contain errors or inconsistencies.

Scope and Delimitations

Salmonellosis outbreaks are zoonotic and associated with meat products and egg-based dishes (Gould et al., 2013). However, Outbreaks can result from fresh fruits and produce (Hanning et al., 2009). This study is focused on salmonellosis caused by fresh produce alone and not animals. This study is restricted to the state of Maryland, and findings may not be generalized beyond the state. This study involved use of secondary data limited to findings from the Maryland Department of Health and Foodborne Disease Active Surveillance Network.

Limitations, Challenges, and Barriers

Some national foodborne outbreaks of salmonellosis are underreported in data compiled by counties and state health departments. Surveillance data are dynamic because data from previous years may vary as isolates are added or corrected (CDC, 2011). There is a strong likelihood of many missing cases. Not all persons with a *Salmonella* infection seek medical care; therefore, there is no way to account for victims in this category via national surveillance (Scallan et al., 2006). Laboratory identification of *Salmonella* isolates, variable sources, and origins can pose possible challenges or barriers to this study. Once a particular foodborne salmonellosis outbreak is suspected, stool samples from affected persons are transferred to diagnostic laboratories of the U.S. Department of Agriculture (USDA), FSIS, Animal and Plant Health Inspection Services, and National Veterinary Services laboratories for proper serotyping. Possibility of information bias due to my occupational affiliation with the FSIS in one of Maryland's counties did not influence the study's outcomes since the data I used were obtained from secondary sources.

Significance

According to Scharff (2012), the economic cost of foodborne illnesses in the U.S. is $77.7 billion annually. This number encompasses medical costs, productivity losses, illness-related deaths, pain, suffering, and functional disability. Cases of infection and occurrence continue to soar. According to the CDC (2014), there were 7439 incidences and 2144 hospitalizations, with 32 deaths in the U.S. due to *Salmonella* alone in the year 2014. This study clarified the influences of agents and locations of transmission, seasonal variability, and demographic characteristics of affected populations in terms of the occurrence of *salmonellosis*. The system thinking approach was used to offer suggestions for statewide implementation of preharvest practices, including good agricultural practices and postharvest measures such as the HACCP plan. Also, findings from this study can help improve strategies for pre and postharvest contamination prevention interventions.

Social Change Implications

One positive social change implication of this study is that it will explain to produce farmers, consumers, and local community health educators mechanisms of transfer of *Salmonella* through the produce supply chain so they can help prevent contamination of produce during either the preharvest or postharvest stages of production. Also, knowledge gained from this study will help reduce the burden of foodborne diseases and increase awareness of inspection officers to issues related to the food chain. Local food safety policy makers can integrate the system thinking model into more functional prevention guidelines that can improve farm-to-fark food safety for Marylanders. Furthermore, eco-friendly sanitizer application during postharvest washing of produce before transportation and immediate introduction of the HACCP plan after delivery to restaurant or consumption outlets will enhance produce safety for all Marylanders. Accurate temperature control of less than 41 F is a Code of Maryland Regulations (COMAR) requirement for cut fruits and vegetables during transportation and storage of fresh produce in Maryland and should be part of the HACCP plan.

Also, washing of raw fruits and vegetables to remove soil and other contaminants with clean water or approved sanitizer before cutting or serving is essential to break mechanisms of salmonellosis transfer. There is the possibility of transferring *Salmonella* along the food supply chain if thorough washing is not done at the food service facility level before cutting and serving or packaging.

According to Hanning (2009), several produce items specifically have been identified in outbreaks, and the ability of *Salmonella* to attach to vegetables and fruits may be factors that make these produce items more likely to be sources. Foodborne *Salmonella* is also traceable to zoonotic sources like intestinal tracks of animals including reptiles, amphibians, live poultry chickens, ducklings, geese, and turkeys that may pass them out in their feces into the environment as well as run-offs (Behravesh et al., 2014). Manure, irrigation water, and run-offs are integral parts of environmental ecosystems which grow fresh produce, resulting in contamination of the produce either during the preharvest or postharvest stages.

Summary

Understanding the effects of pathogens in consumer food safety as well as their types, mode of transfer, symptoms, and prevention continues to be of utmost concern to public health. Inflammation of the stomach and intestines manifests with symptoms such as fever, abdominal pain, diarrhea, nausea, and vomiting and has an incubation period of 12 to 72 hours, lasting for 4 to 7 days (Kovacic et al., 2017). According to Liu et al. (2018), *Salmonella* accounts for 53.5% of all foodborne disease outbreaks, 32.7% of which are associated with consumption of produce.

This quantitative study involved assessing relationships between occurrence of salmonellosis and consumption of fresh produce, demographic characteristics of the study population (residents of the state of Maryland), seasonal variation, sites of occurrence, and types of produce consumed. I used the FSMA traceability template as a context for my study. Secondary data for this study were obtained from the epidemiological unit of the MDH. Chapter 2 includes a literature review involving rates of incidence, demographics, seasonal variation, geographical distribution, outbreaks, antimicrobial resistance, produce items, and environmental sources.

Chapter 2: Literature Review

Introduction

The purpose of this quantitative study was to investigate major risk factors in salmonellosis outbreaks in Maryland, methodically evaluate their influence on the consumption of raw produce (predictor variable) and determine the relationship between foodborne salmonellosis (dependent variable) and the source of contamination. There is increasing evidence that the *Salmonella* pathogen can survive in both wild and domestic animals as normal gastrointestinal microflora in reservoirs, spillover hosts, or dead hosts (Persad & LeJeune, 2018). This resident enteric flora of animals is shed in animal feces, but the exact etiology of these cases is still unknown (Liu et al., 2018; Persad & LeJeune, 2018). Incidence of foodborne salmonellosis via the consumption of fresh vegetables, leafy greens, and other raw produce with minimal cooking is also increasing. However, pre- and post-harvest intervention strategies for decontamination of fresh produce are still ineffective (Herman et al., 2015). The contamination of fresh produce that is consumed raw is traceable to the pre- and post-harvest stages of the food supply. This new challenge calls for more surveillance to be directed towards the pre- and post-harvest stages of the food supply for adequate intervention techniques. Therefore, surveillance can be achieved if the geographical distribution of implicated food items and characteristics of pathogens are identified and traced to the source of any given outbreak.

Furthermore, as sources of incidence of salmonellosis outbreaks shift due to consumers' choices from traditional sources such as poultry, dairy, and animal products to fresh produce, so is the need of compliance officers for regulated surveillance.

Contaminated vegetables and fruits or processed plant products and their consumption have resulted in recent salmonellosis outbreaks, like the 2011 outbreak in Germany due to the consumption of mung beans sprouts (Abu et al., 2012). The adaptation of *Salmonella* to the plant habitat may not seem entirely natural. However, the ecology of plant-associated bacteria has a limited survival rate (Kozak et al., 2013). It renders areas with high-density animal production to be considered high-risk sources for the survival, transport, and contamination of raw produce (Jacobsen & Bech, 2012). The introduction of various farming techniques, globalization of food production and supply, and increasing demand for convenient food items has resulted in trends involving produce-based outbreaks and an increasing need for the CDC to survey the incidences of foodborne diseases (Kozak et al., 2013). According to Bush and Perez (2018), 30% of untreated patients, referred to as chronic enteric carriers, harbor organisms in their gallbladder and shed them in the stool for greater than 1 year. There is evidence that most of the estimated 2000 carriers in the US are older women with a chronic biliary disease.

Summary of Literature Establishing Relevance of the Problem

Salmonella is notable among pathogens responsible for foodborne outbreaks in fresh produce. According to Liu et al. (2018), *Salmonella* accounts for 53.5% of all foodborne disease outbreaks, 32.7% of which are associated with the consumption of fresh produce. Wills et al. (2015) said about 2.2 million people die annually from both foodborne and waterborne diseases.

Climate change has contributed to salmonellosis outbreaks. *Salmonella* pathogens have a direct positive correlation with ambient temperatures and rapid replication, manifesting during warmer temperatures due to global warming than any other time of the year (Akil et al., 2014). A decline in glaciers in both hemispheres shows a direct consequence of global warming. There has been a noticeable global temperature increase of 0.74°C between 1906 and 2005, and a corresponding yearly rise of two millimeters per year in sea level, resulting in a 7.4% decline in Arctic Sea ice (Mills et al., 2010). Changes in weather patterns, especially precipitation and wind patterns, are capable of moving tons of matter, either in solid, liquid, or gaseous states, and also move viruses and bacteria pathogens within the environment. Weather patterns are a contributing factor in terms of the transfer of microbial contaminants to leafy vegetables and raw produce, particularly dust particles that settle on leafy vegetables during dust storms (Akil et al., 2014). Contamination in the U.S. is due to irrigation water, handling procedures, runoff from nearby animal feedlot operations, and farm workers' waste disposal practices due to a lack of adequate facilities (Iwu & Okoh, 2019).

The permeable nature of the external surface structure of fresh produce can encourage biofilm formation that can serve as a harborage for pathogens. According to Colle (2015), anatomical and morphological changes during the development of fruit surfaces play a role in plant development and provide protection against abiotic and biotic factors such as pathogens. Pathogens do not need to gain access to cells to survive or spread, but can colonize surface barriers forming biofilms that can transmit organisms. Biofilms can be on surfaces of raw produce, submerged surfaces, conduits in processing equipment, air-liquid interphases, or any suitable environment conducive for pathogens to grow on (Wu et al, 2012). Fresh produce become common hosts when pathogens abandon a planktonic single-cell existence in an aqueous environment to form multicellular colonies by adhering to available solid surfaces on produce (Claessen et al., 2014). These bacterial pathogens have been found to contaminate fresh produce on surfaces like leafy greens, alfalfa sprout, cantaloupes, watermelon, and lettuce (Delaquis et al., 2007; Doering et al., 2009; Fan et al., 2009). The world involves increasing international travel and food trade, and this has given rise to greater interdependence among nations in terms of food safety, surveillance, and regulations. Therefore, the regulatory agencies' need for maintaining an epidemiologic database to keep track of possible outbreaks becomes very important.

Preview of Major Sections of the Literature Review

In this chapter, I established the purpose and justification for this study. I addressed strategies for searching literature, the theoretical foundation and conceptual framework under study, a literature review detailing characteristic of *Salmonella*, and justification for the inclusions of some variables and concepts.

Literature Search Strategy and Terms

In the literature review, I used Walden University Library resources to access Google Scholar, ProQuest, SAGE Journals, and EBSCOHost. Government databases were accessed from organizations including the National Environmental Health Association, CDC, FDA, USDA, National Population Commission (NPC), FEMA, US Environmental Protection Agency (USEPA), and FoodNet. Literature was searched that was published between 2017 and 2021. I used the following search terms: *salmonellosis*, *seasonality of Salmonella*, *food safety magazines*, *clinical manifestation of* *salmonellosis*, *food processing*, *salmonellosis outbreaks in the United States due to fresh produce, sources and potential intervention measures, comparing human-Salmonella with plant-Salmonella protein-protein interaction predictions, foodborne salmonellosis, preventing foodborne diseases through handling, recent foodborne diseases and outbreaks,* and *environmental effects on foodborne outbreaks in Maryland*. My focus was peer-reviewed articles and Walden University dissertations.

Theoretical Foundation

In this study, I used the system theory to identify both interacting and interdependent components of the food supply chain, namely growers (farmers), shippers (transporters), retailers (grocery stores), and the food service industry (restaurants). Von Bertalanffy developed system thinking and referred to it as a complex of interacting components and concepts characteristic of organized wholes such as interaction. Similarly, the food supply chain model is based on the system theory approach to food supply.

According to Kramer and de Smit (1977), system thinking is a way of looking at reality in terms of wholes, acknowledging that the environment is an essential part of the system while interacting with that system. Contemporary system thinkers like Wheatley (2006), von Bertalanffy (1968), Wilber (2000) and Meadows (2009) all posit a holistic approach to solving complex problems in an organic world with the assumption that everything is interrelated. System thinking has evolved through the years from a single disciplinary tradition, as was the case at inception in the 20th century through evolving inter-disciplinary disciplines. It has crossed between boundaries enabling research through a wide variety of stakeholders to trans-disciplinary tradition (Peters, 2014).

Modeling and Recent Application of System Thinking

The concept of system thinking requires a good understanding of causal relationships and feedback based on the principles of an organization using systematic or holistic thinking (Haraldsson, 2004). It is a science of interconnectedness between entities, without isolating any unit as an entity. The application of system thinking is successful only when there is a balance between system analysis (SA) and system dynamics (SD) when using a given model.

Health planners used a recent application of this theory in Austin, TX, where system dynamics model (SD model) was used to align prevention efforts in tackling cardiovascular disease risk with maximizing the effect of limited resources (Loyo et al., 2013). Using the SD model requires coordinating resources with the best of intentions and simulating how they flow through with expected results. The Austin Health planners used the available evidence of disease prevalence (cardiovascular disease), risk factors, local contextual factors, resulting in health conditions, and impact on population health to achieve results (Loyo et al., 2013).

System thinking can be applied to the food supply chain by bringing all complex social, managerial, economic, and ecological systems into a functional model with interconnecting factors (Merrill et al., 2013). A model, in this case, is simply a casual loop diagram connecting each component of the food supply chain. Food supply involves a whole supply chain, from farm-to-fork, and including the processes of planting, cultivating, processing, transportation and handling, storage, preparation, and consumption. The HACCP plan procedures and standardizations are methods put together to reduce and control sources of food contamination. According to Trbovich (2014), “system theory centers on the dynamic interaction, synchronization, and integration of people, process, and technology” (p. 31). Food supply, like health care, involves a whole process known as the supply chain from farm-to-fork. It starts with the food chain, i.e., the planting, cultivating, processing, transportation, handling, storage, preparation, and consumption of food. Also connected to the food chain, with the advancement in technology in food production, is the selection of the type of soil, irrigation system, post-harvest washing before packaging.

The rising global dataset on *salmonellosis* and related foodborne illnesses points to the need for prevention strategies, more robust surveillance and regulatory measures, food safety, and health education (Käferstein et al., 1997). In epidemiologic surveillance techniques, attention should be focused on risk assessment, hazard identification, hazard characterization, exposure assessment, and the challenges of embarking on food safety surveillance (Käferstein et al., 1997).

Conceptual Framework

Contaminated produce is traceable to the pre- and post-harvest stages of food supply and continues to pose new health challenges (Hanning et al., 2009). Therefore, more surveillance needs to be directed towards the entire process for adequate intervention. The adaptation of *Salmonella* to the plant habitat may not seem entirely natural. However, plant-associated bacteria can survive for a limited time in the environment (Kozak et al., 2013). More so, the areas with high-density animal production are considered high-risk sources for pathogen contamination of raw produce (Jacobsen & Bech, 2012). The present-day sophisticated farming techniques, globalization of food supply network, and the craving or taste for convenience food items has created the need for increased surveillance for foodborne disease incidences (Kozak et al., 2013). The COVID-19 pandemic is a typical example of how a disease pathogen can start from any part of the world and, if not controlled, quickly become a global disease. The introduction of various advanced farming techniques, globalization of food production and supply, and the taste for convenience food items have all combined with the need for increased surveillance of foodborne disease incidences to catch up with the trends of produce-based outbreaks (Kozak et al., 2013). According to a review in the Merck Manual, about 30% of untreated patients, referred to as chronic enteric carriers, harbor organisms in their gallbladder and shed them in the stool for >1yr. There is evidence that most of the estimated 2000 carriers in the US are older women with a chronic biliary disease (Bush & Perez, 2018). This population is another possible source of contamination of produce.

Antimicrobials

Public Health is preventative and proactive and because *Salmonella* exhibits the ability to develop antibiotic resistance, it becomes essential to identify the specific risk factors for infection and the raw produce implicated in an outbreak (Crim et al., 2018). In a surveillance study to determine the resistance of Newport serotype, which is estimated to cause a 1.2 million human *salmonellosis* cases annually, 88% of *Salmonella entrica* isolates were susceptible, and 8% were resistant to all antimicrobials (Crim et al., 2018). Therefore, there is an increasing need to harness all available resources towards addressing this public health challenge in specific areas like surveillance, diagnosis, treatment, and prevention of possible outbreak. One goal of the study is seeing the entire system as one "whole", subsequently, attention may be focused on any particular subset (surveillance, diagnosis, treatment, and prevention) that needs more attention than the other at any point in time. Essential components of the study included incidence, demographics, seasonal variation, geographical distribution, outbreaks, and antimicrobial resistance, produce items, and environmental sources (Crim et al., 2018). The *Salmonella* pathogen has been found to attach to or internalize into vegetables and fruits, from sources like contaminated water or surface runoff used for irrigation and washing of produce (Hanning et al., 2008). Surface runoff from rain events carries animal wastes containing a mixture of microbial pathogens with different antibiotic resistances. Rain events can result in the deposition of pathogens on fruits and vegetables. The *Salmonella* pathogen has been extensively researched. This study used that research knowledge in the understanding of the ubiquitous nature of their ecology, the dissemination pathways and optimal method of control (Hanning et al., 2008).

Biofilm Formation

According to Galié et al. (2018), biofilms are complex microbial ecosystems formed by one or more microbial species immersed in an extracellular matrix of different composition depending on the type of food manufacturing environment and the colonizing species. The number of microbial species in each colony sometimes determines their ability to cling to the surface of the fresh produce or food processing equipment. Both the microbial colony and the nature of the biofilm surface influence their resistance to disinfectants such as the quaternary ammonium compounds (Meyer, 2015).

Biofilm is the formation of an extracellular matrix of a cyst-like unit of microrganisms or fungi that is attached to a food processing or food contact equipment surface (Galié et al., 2018). The biofilm environment can be resistant to disinfectant and difficult to control (Meyer, 2015). The term mechanism is also applicable to biofilm, particularly following the food flow from farm-to-fork. However, the actual mechanism starts from the particular bacterial or fungal species and their colonization, maturation, and the release of biofilm to the food matrix (Galié et al., 2018). Biofilm is a phenomenon that thrives well in a warmer environment.

A biofilm is formed when a pathogenic bacterium colonizes a surface, a piece of equipment, or environment and is resistant to physical or chemical cleaning. Biofilms can be resistant to antimicrobials and sanitization procedures and are capable of cross-contaminating food production (Coughlan et al., 2016). Biofilm can grow on the surface of raw produce, a submerged surface, conduits in processing equipment, the air-liquid interphase, or any suitable environment that provides a substratum for colonization (Wu et al., 2012). Fresh produce becomes contaminated when waterborne pathogens adhere to and colonize plant surfaces (Claessen et al., 2014). Bacterial pathogens have been found to contaminate fresh produce surfaces such as leafy greens, alfalfa sprout, cantaloupes, watermelon, lettuce (Delaquis et al., 2007; Doering et al., 2009; Fan et al., 2009).

Next-Generation Technology

PulseNet is applying the most recent technology to identify and characterize *Salmonella* and related bacteria to prevent possible disease outbreaks. The process of whole genome sequencing (WGS) is a laboratory procedure to characterize or determine the order of bases (A, T, C, and G) in the genome of an organism or genetic material of an organism (bacteria, virus, orange, humans, and animals) (CDC, 2016). PulseNet is utilizing the next-generation sequencing (NGS) technology to develop, evaluate, and implement tools needed for data analysis (CDC, 2016). It is an incredible state-of-the-arts technology for DNA sequencing of bacterial fingerprints. Using this technique, scientists can apply pulsed-field gel electrophoresis (PFGE) to produce a DNA fingerprint to investigate bacterial isolates from sick people, contaminated food, the food source, and process (CDC, 2016).

Evidence of Plants as Bona Fide Hosts for *Salmonella*

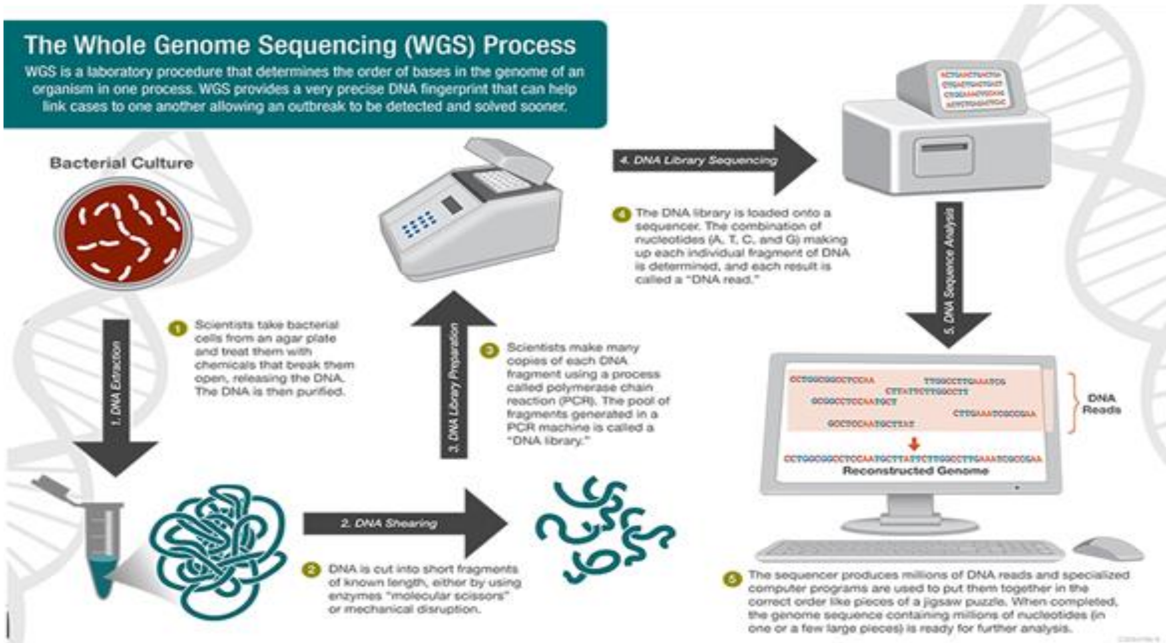
There continues to emerge evidence that *Salmonella* interacts with plant cells. Like in the mammalian cells, *Salmonella* utilizes the same functional protein mechanism known as TTSS-1 and -2, which can suppress the plant host immunity (Schleker et al., 2015). There are links between fundamental cellular metabolism and the chemical pathway leading to secondary metabolite synthesis. There is consistent evidence that gastrointestinal infection with bacterial pathogens is positively correlated with ambient temperature, as warmer temperatures enable more rapid replication. Warming trends in the United States and specifically in the southern states, may increase rates of *Salmonella* infections (Akil et al., 2014).

Mechanism of Contamination Transfer

Recent studies link the salmonellosis outbreak due to contaminated raw vegetables and fruits to food poisoning (Wiedemann et al., 2015). While the origin of contamination can no longer be limited to a point source, a salmonellosis outbreak in the farm-to-fork program is possibly traceable to more than one source of contamination.

Figure 2

Whole Genome Sequencing



Retrieved from: CDC. (2016). Pulsed-field gel electrophoresis https://www.cdc.gov/pulsenet/pathogens/pfge.html

However, the interactions and pathogenicity of *Salmonella* on a cellular level become possible through the putative protein-interactions (PPI), where the Salmonella-human host transfers the corresponding molecular interactomes to the *Salmonella*-plant system (Schleker et al., 2015).

Stratified geopolitical sampling was conducted between 2010 and 2011, and a total of 412 tomato farms were sampled, with 135 composite samples per state from Kaduna, Kano, and Katsina (Shenge et al., 2015). Also, 100 ml of irrigation water was collected from each source contributing to the irrigation and stored in sterile sample bags. This study identified several post-harvest contaminated tomato fruits in the hub of Nigerian fresh produce zone. The study also identified several preharvest sources of tomato contamination within the catchment states (Shenge et al., 2015).

An essential strength of the Shenge et al. (2015) study is to identify critical areas of cross-contamination, i.e., animal manure, irrigation water, and tomatoes. To undertake this cross-over from one host to the other requires diversification of both the ruminant animals (cattle, sheep, and goats) and plant host system. These diversified interactions go through a stage-wise process dependent on *Salmonella* interaction with its host and are controlled by the following factors: (i) attachment to host surfaces; (ii) entry process; (iii) multiplication; (iv) suppression of host defense mechanism. However, this aspect of this study involves pointing out both the similarities and differences between animal and plant infections (Wiedemann et al., 2015).

Justification for Selection of the Variables or Concepts.

Raw produce contamination is a widespread experience for tomato farmers in Nigeria. The associated fecal indicator organism counts on fruits and vegetables that is as high as 2.45 Log MPN/g by surface runoff in the raining season, drops down to 1.10 Log

MPN/g during the dry season (Shenge et al., 2015). While this Nigerian study points to the danger of surface runoff during weather activities, it is also important to visualize the possible pathways of contamination of this unique ecology.

Temperature variation had an impact on the Nigerian study. There are two seasons in Nigeria; the raining season that starts May through September and the dry season which starts from November through April. Remarkably, climate patterns in the ecosystem play a role on the dynamics of pathogens in the environment. The weather pattern has been the enabling reason for the transfer of microbial contaminants to leafy vegetables and raw produce, particularly dust particles that settle on leafy vegetables during dust storms (Akil et al., 2014).

The preharvest stage of the ready-to-eat (RTE) produce includes the choice of irrigation water and the nature of the agricultural soil structure, which can pose a problem if contaminated with pathogens. In contact tracing, therefore, the entire agro-ecosystem process must be considered and a careful understanding of the stepwise processes of pre- and post-harvest is necessary for effective intervention (Iwu & Okoh, 2019). The emergence of antibiotic-resistant *Salmonella* can be a troubling challenge to the biochemical science of sanitizing resulting in resistance by different isolates of *Salmonella* (Olaimat et al., 2018). This will pose a significant threat to the food industry, particularly the raw produce used in various salads that constitutes the ready-to-eat (RTE) menus under the farm-to-fork program (Olaimat et al., 2018).

Summary and Conclusions

Usually, people infected with *Salmonella* take about 12 to 72 hours to develop symptoms to a particular strain of these bacteria which last for 4 to 7 days before clearing naturally or when treated with antibiotics (CDC, 2019). A traceback investigation is always a stepwise approach to ensuring that an outbreak receives the quickest attention to curtail further spread, to prevent future incidence like the most recent multistate outbreak that was traceable to cut fruit, including melon, cantaloupe, pineapple, and grapes (CDC, 2020). Facilities responsible for any outbreak must be held accountable so that they might exercise some caution when handling potentially hazardous food items that may result in an outbreak. From my personal experience when conducting HACCP inspections, most food service facilities (FSF) are simply reckless in their pursuit of quick money.

A very important component of the Crim et al. (2018) study included incidence, demographics, seasonal variation, geographical distribution, outbreaks, and antimicrobial resistance, produce items and environmental sources. The *Salmonella* pathogen has been extensively researched; so is the research information about the ubiquitous nature of their ecology, the importance of the dissemination pathways, and optimal method of control remains a concern to public health (Hanning et al., 2008). The effects of salmonellosis outbreaks in consumer food safety, their types, mode of transfer, symptoms, and prevention are major public health concerns in the State of Maryland. Fresh produce such as Berries, Cantaloupe, Herbs, Lettuce, Spinach, Sprouts, and Watermelon have been implicated in and found to be responsible for several types of gastroenteritis. However, the major risk factors for the development of salmonellosis and their relationship to the consumption of raw produce in the state of Maryland are not well understood. Hence the need for this study.

In the next chapter, the research design, justification for its choice, description of the study area, data sources, and how the data was collected were clearly stated. Also, Chapter 3 includes a description of the types of variables, their measurements, operationalization, and how the data were analyzed, both internal and external validity of the study were discussed, and ethical concerns.

Chapter 3: Research Method

Introduction

Outbreaks of salmonellosis traceable to fresh produce consumption in Maryland from 2010 through 2018 and major risk factors associated with these outbreaks was the primary focus of this study. Also, the study was designed to explore epidemiological concerns involved with these outbreaks such as agents of transmission, locations of outbreak, and seasonality.

This chapter includes descriptions of the research design for this study and justification for its choice. Descriptions of the study area, data sources, and how data were collected are also addressed in this chapter. The chapter also includes a description of types of variables, measurements, operationalization, and how data were analyzed. Potential threats to the study involving internal and external validity were discussed along with ethical concerns. This is followed by a summary of research methods, which served as a transition to Chapter 4.

Research Questions and Hypotheses

In this study, I addressed relationships between salmonellosis outbreaks due to fresh produce, risk factors involved in this outbreak, and those at high risk of contracting the disease in Maryland. The following are RQs this study addressed:

RQ1: Is there an association between the consumption of fresh produce and the occurrence of salmonellosis?

$H_0 1$: There is no association between the consumption of fresh produce and the occurrence of salmonellosis.

H_a1: There is an association between the consumption of fresh produce and the occurrence of salmonellosis.

RQ2: Is there an association between demographic characteristics (age, sex, race and ethnicity) of Maryland and the risk of contracting salmonellosis through the consumption of fresh produce?

H_02: There is no association between demographic characteristics of Maryland and the risk of contracting salmonellosis through the consumption of fresh produce.

H_a2: There is an association between demographic characteristics of Maryland and the risk of contracting salmonellosis through the consumption of fresh produce.

RQ3: Is there an association between salmonellosis foodborne outbreaks from fresh produce and seasonal patterns in Maryland?

H_03: There is no association between salmonellosis foodborne outbreaks from fresh produce and seasonal patterns in Maryland.

H_a3: There is an association between salmonellosis foodborne outbreaks from fresh produce and seasonal patterns in Maryland.

RQ4: Is the association between consumption of raw produce and development of salmonellosis modified by geographic location in Maryland?

H_04: The association between consumption of raw produce and development of salmonellosis is not modified by geographic location in Maryland.

H_a4: The association between consumption of raw produce and development of salmonellosis is modified by geographic location in Maryland.

RQ5: Is the association between salmonellosis outbreaks and fresh produce consumption modified by the type of produce consumed?

$H_0$5: The association between salmonellosis outbreaks and consumption of fresh produce is not modified by the type of produce consumed.

H_a5: The association between salmonellosis outbreaks and consumption of fresh produce is modified by type of produce consumed.

Research Design and Rationale

This quantitative study involved assessing relationships between salmonellosis outbreaks due to fresh produce, risk factors involved in outbreaks, and those at high risk of contracting the disease in Maryland. RQs addressed associations between different independent variables and a dependent variable. As such, quantitative research is an appropriate research design for this study. This is because the study involved testing different proposed hypotheses and assessing various associations and predictive abilities between independent and dependent variables and the strength of such associations.

By contrast, in qualitative research, the main interest of the researcher is to understand a phenomenon rather than predict future actions by proposing and testing hypotheses for generalizable outcomes (Bendassolli, 2013). Qualitative studies are used to identify and explore human issues but not suitable for investigating problems with statistical or numerical origins (Onwuegbuzie & Byers, 2014). Mixed methods was not suitable as it is used to produce findings that involve both qualitative and quantitative methods.

A cross-sectional study design was used for this study. This is a type of observational study where both exposure and outcome can be measured at the same time. It is defined as a snapshot of the study population at a given time. RQs for this study require one-time evaluations of different independent variables as well as the dependent variable. Thus, an experimental research design was not suitable for this study.

Considering the stated research questions and hypotheses for this study which described the relationships between the different independent and dependent variables, this did not require comparison assessing the effects of an intervention as would have been for an experimental or quasi-experimental design. An advantage of the cross-sectional design is that it has an immediate outcome assessment. It is also ideal for research participants scattered over a wide geographical area.

Secondary data analysis was used for this study. Secondary data are data that have already been collected and analyzed by someone else for another primary purpose (Johnston, 2014). Therefore, secondary data analysis involves re-analyzing, interpreting, and the reviewing of past data for a new research inquiry (The Oxbridge Research Group, 2020). Research usually begins with an enquiry on what is known, and what is yet to be known about a topic. In conducting research, the study area and the research questions to a large extent determine the research method to be employed (Cresswell, 2009). Data may already exist that may be adequate to answer the research questions. This is advantageous as it reduces time, costs and it is much easier as the researcher is less involved with the logistics of collecting data. Secondary data analysis for research is a systematic method with the following procedural steps: the development of the research questions, identifying the dataset(s), evaluation of the dataset for reliability, suitability and adequacy. This is then followed by the analysis of the secondary data in line with chosen statistical tests to answer the research questions (Johnston, 2014).

Methodology

Study Area

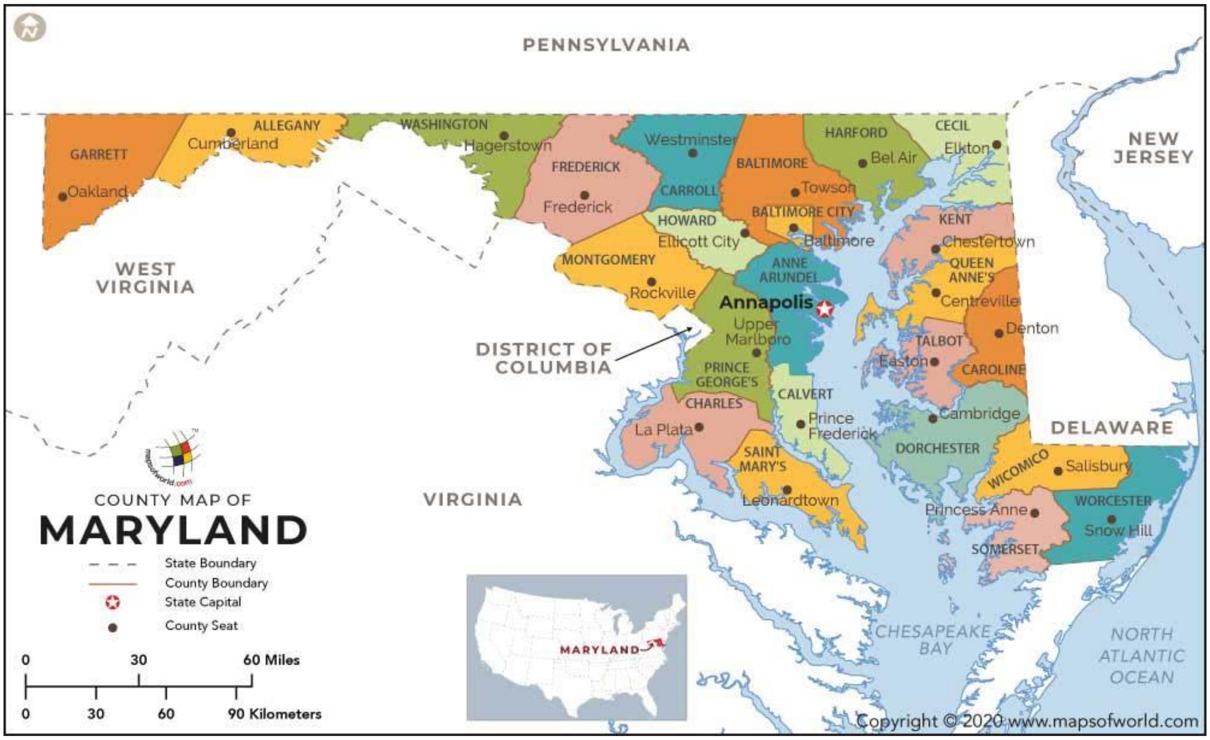
This study covered the state of Maryland in the United States of America. Maryland, one of the 50 US states, is located in the Mid-Atlantic region in the northeast of the United States. It is situated in the rich natural estuary of the Chesapeake Bay, bounded on the north by the State of Pennsylvania, west by West Virginia, south by Virginia, and east by Delaware. With an area of 32,131 km^2, Maryland is ranked 42nd of the 50 US states in size. The state is made up of 23 counties (United States Census Bureau, 2019).

Study Population

According to Frankforth-Nachmias and Nachmias (2008), a population can be defined as an aggregate of cases with common characteristics. The population for this study will be the residents in the State of Maryland. The population of Maryland is 6,045,680 and broken down into major racial groups and is comprised of White 56.19%, Black, or African American 29.78%, Asian 6.23%, Hispanic or Latino 7.2%, and Native American 0.6% (United States Census Bureau, 2019). Male and females, young and old of all races and ethnicity will be considered in this study.

Figure 3

County Map of Maryland



Sampling and Sampling Procedures

Data for my study were obtained from a statewide population-based surveillance system conducted by both the local and state public health officials and released by the MDH. To collect surveillance data, once a complaint for possible foodborne outbreak is made to the local Health Department in MDH, a case determination was done using the Gastroenteritis Case Report Form developed by the CDC (see Appendix B). The sampling for *Salmonella* and other foodborne pathogens outbreaks in Maryland Cities and Counties is conducted by the local Public Health officials, Environmental Health Specialist, or Environmental Sanitarians. The local health departments share results and outcomes of investigations with the MDH. However, in the event an outbreak spreads across cities, counties, or the entire state, then the CDC who routinely collaborates with the FDA or Food Safety and Inspection Service (FSIS), part of the U. S. Department of Agriculture are involved throughout all phases of outbreak investigation (CDC, 2019).

Frankforth-Nachmias and Nachmias (2008) stated that sampling is a process whereby a specified number of elements are selected from a sampling frame which is the actual list of the possible elements in the population. Generalizability of the sample to the study population greatly depends on the representativeness of the sample and the extent to which the units of the sample have the same characteristics as the population from where they are drawn. For this study, all the 23 counties of the State of Maryland were included. This ensured representativeness of all the regions of Maryland and improved the generalizability of the study findings. Since the study is a secondary data analysis, there was no time constraints or logistic restrictions to the accumulation of data.

Sample Size Analysis

The sample size for this study is determined by the use of G*Power 3.1.9.4 statistical analysis tool (Faul et al., 2009). The reason for sample size determination is to get an adequate number of study units capable of reflecting unknown parameters after data collection. Sample size determination is influenced by some factors which include the aim of the study, the size of the population and the acceptable sampling error (Pourhoseingholi et al., 2013). Other factors that need to be considered for sample size determination are the number of predictors, the effect size, the alpha level, and the statistical power (Singh & Masuku, 2014). G*Power 3.1.9.4 statistical analysis tool is a power test of the probability of rejecting a null hypothesis when it is actually false (Faul et al., 2009). The OR of 1.3 was chosen as a default parameter from the G*Power software used for the calculation. Also, the OR can be algebraically determined by calculating the relationship between the exposed cases (a/b) and unexposed cases (c/d) from the base population. To calculate the minimum sample size that adequately powered this study, the power of analysis was set at 0.95, the alpha level at 0.05, and a normal distribution for the population, the sample size was 1188. The effect size is a standardized measure and strength of relationship between variables that will answer the research questions on a numerical scale (Pek & Flora, 2017; Schafer & Schwarz, 2019). Since the study used archival data, there were no problem with response rate. Therefore, the minimum expected required size was **1188** (see Figures 3 and 4). However, being a secondary data analysis, a census was done as all the available entries in the database were used.

Secondary Data Evaluation

The secondary data for this study was obtained from the epidemiological unit of the Maryland Department of Health. A Data Use Agreement to use the database is in Appendix A. The Maryland Department of Health is one of 10 US States Health Departments, along with the US Department of Agriculture's Food Safety and Inspection Service, and the U.S. Food and Drug Administration which forms the Food-foodborne Disease Active Surveillance Network (FoodNet). FoodNet is an active surveillance network in collaboration with the CDC for the national surveillance of gastroenteritis, C*ampylobacter* infections, salmonellosis, shigellosis, and other foodborne diseases (CDC, 2020).

The Maryland *Salmonella* surveillance data is collected by the officers in the Epidemiologic Unit of Maryland Department of Health using a standard survey instrument developed by the CDC (Appendix B). This data was collected by surveillance of laboratory confirmed human *Salmonella* isolates. Diagnosed *Salmonella* isolates are submitted to the public health laboratories of the department of health for further confirmation and serotyping. Unusual serotypes are further sent to the CDC reference laboratory for further confirmation and characterization from where the results are reported back to the state department of health.

The data collection method was by the use of the survey method, and it is an on-going perennial process. The data set for the years 2010 to 2018 for the state of Maryland is available and was used in this study. The data for the year 2019 is still being finalized (and delayed due to the COVID-19) infections and was not available for use at this time. This data had been de-identified as all information that can lead to breach of confidentiality had been removed before given to me. An assessment of the data set shows that the relevant variables needed to answer the research questions for the study are included. In conclusion, the data is suitable for use in this study as the mode of data collection was standard following nationally approved guidelines.

Types of Variables and Measurement

The variables of interest in this study are dependent and independent variables. Depending on the research question, some of the variables can either be independent or dependent variable. The main dependent variable in this study was the diagnosis of salmonellosis as verified by identification of *Salmonella* from the patient’s stool sample after eating any of the following fresh (raw) produce cantaloupe, spinach, sprout, watermelon, tomatoes, berries, lettuce, and herbs.

Figure 4

*Calculation of Sample Size Using G*Power 3.1.9.4*

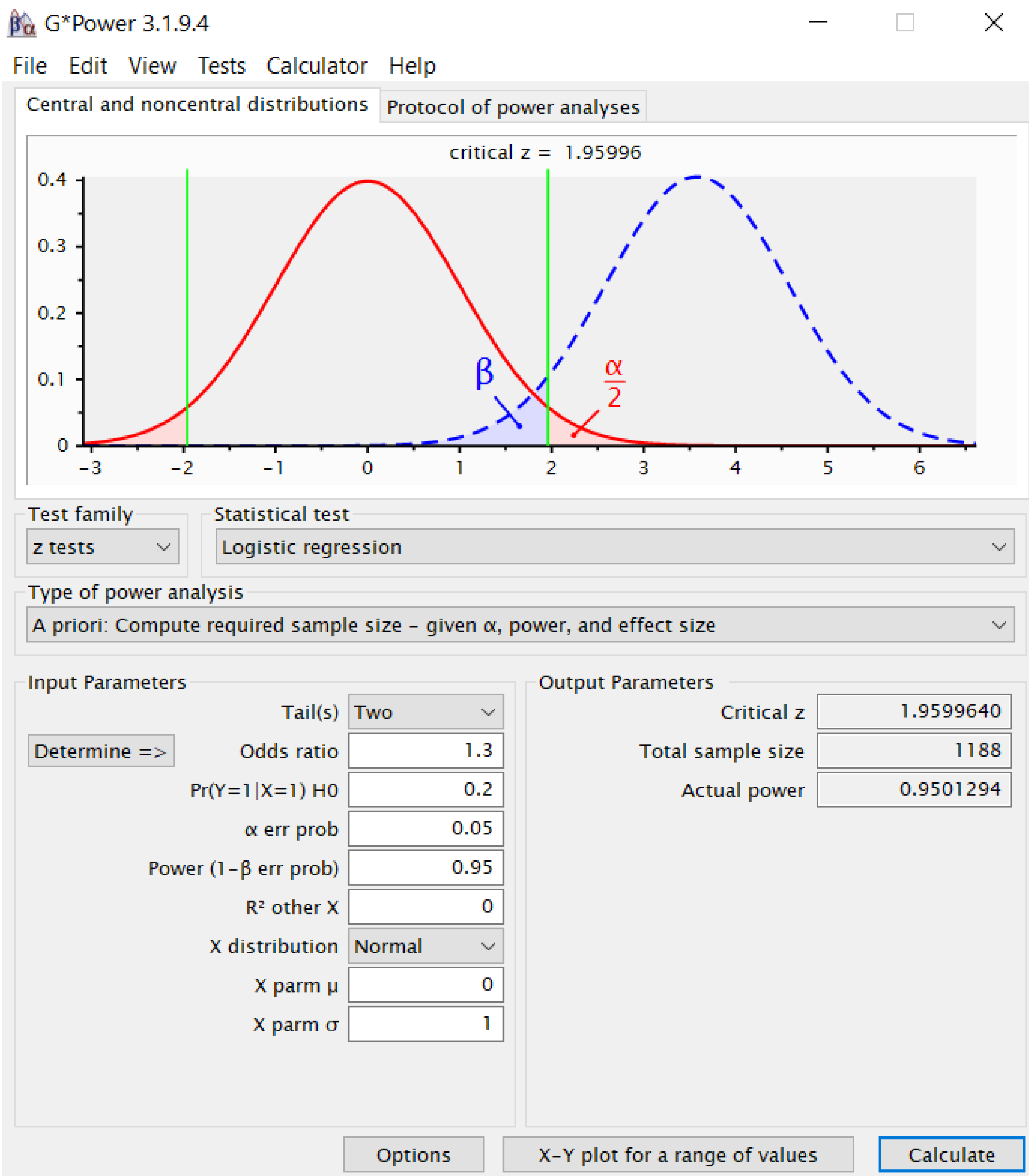
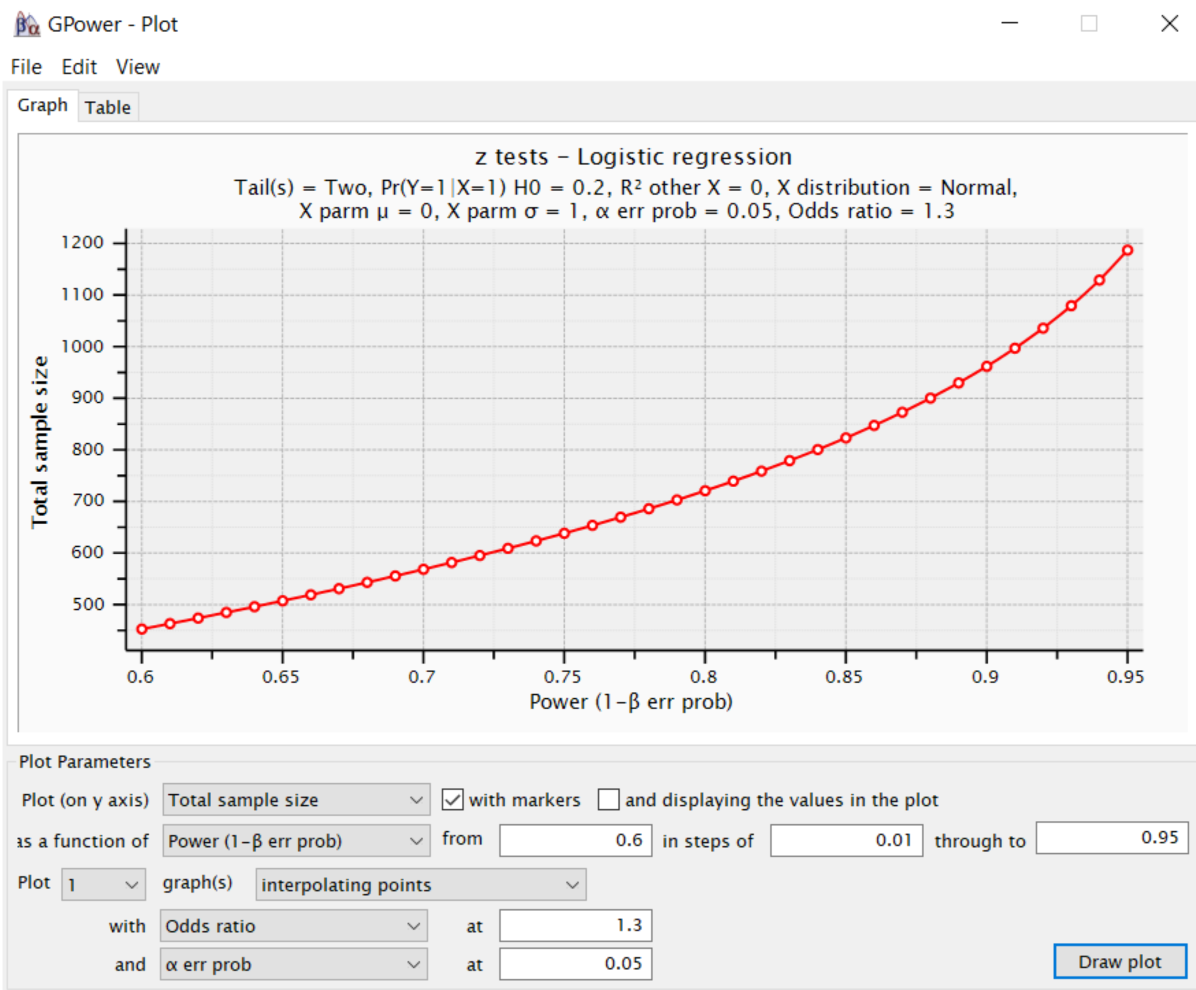


Figure 5

*Graphic Analysis of Sample Size Using G*Power 3.1.9.4*



Other variables considered in this study include the demographic parameters of the study population comprising age, sex, race, ethnicity, and their county of residence; the different locations where food is served that had an outbreak (region, county, and city), seasonal variation of the outbreak, and type of produce consumed (agent of transmission). Table 1 shows the variables of interest and their operationalization.

Table 1

Variables and Operationalization

Hypotheses	Name of Variable	Type of Variable (independent, dependent)	Definition of the Variable (Construct)	How the Variable will be Operationalized (Measured)	Level of Measurement (nominal, ordinal, interval, ratio)
Hypothesis 1	Consumption of raw produce	Independent	History of eating or not eating raw produce	1=Yes 2=No	Nominal
	The occurrence of *salmonellosis*	Dependent	Diagnosis of *salmonellosis* as verified by identification of S*almonella* from the patient's stool sample after eating any of the following: Cantaloupe, spinach, sprout, watermelon, tomatoes, berries, lettuce, and herbs	1=Yes 2=No	Nominal
Hypothesis 2	Age	Independent	Age group of patients at system onset	Interval scale responses accordingly: <5 5 - 9 10 - 19 20 - 64 65+	Interval
	Sex	Independent	Case Patients' sex	1=Male 2=Female 3=Unknown	Nominal
	Race	Independent	Case Patient's race	1=Asian 2=Black 3=American Indian/Alaskan Native 4=Multi-racial 5=Other 6=Pacific 7=White 8=Unknown	Nominal
	Ethnicity	Independent	Case Patient's ethnicity	1=Hispanic 2=Non-Hispanic 3=Unknown	Nominal

	Location	Independent	Case patient's cities, counties and regions		Nominal
	Positive culture of *Salmonella* after eating fresh (raw) produce	Dependent	Identification of *Salmonella* by culture after eating any of the following: Cantaloupe, spinach, sprout, watermelon, tomatoes, berries, lettuce, and herbs	1=Yes 2=No	Nominal
Hypothesis 3	*Salmonellosis* foodborne outbreak from fresh produce,	Dependent	Identification of *Salmonella* by culture after eating any of the following: Cantaloupe, spinach, sprout, watermelon, tomatoes, berries, lettuce, and herbs	1=Yes 0=No	Nominal
	Seasonal patterns in Maryland	Independent	Specimen collection dates grouped	Summer Fall Winter, Spring	Nominal
Hypothesis 4	Consumption of raw (fresh) produce	Independent	History of eating raw produce	1=Yes 0=No	Nominal
	Geographic location cities, counties and regions where food is served	Independent (Covariate)	Cities, counties and regions where food is served	Cities Counties' Regions	Nominal
	The occurrence of *Salmonellosis*	Dependent	Identification of *Salmonella* by culture after eating any of the following: Cantaloupe, spinach, sprout, watermelon, tomatoes, berries, lettuce, and herbs	1=Yes 0=No	Nominal
Hypothesis 5	Occurrence of *Salmonellosis*	Dependent	Identification of *Salmonella*	1=Yes 0=No	Nominal

The type of raw produce consumed	Independent	Different types of produce	Cantaloupe Spinach Sprout, Watermelon, Tomatoes, Berries Lettuce Herbs	Nominal
Consumption of fresh produce	Independent	History of eating or not eating raw produce	1=Yes 0=No	Nominal

Data Analysis Plan

The secondary data which was sourced on a Microsoft Excel spreadsheet was entered into the computer and analyzed with statistical package for social scientists (SPSS) software version 27. I am also in possession of the data dictionary used for the coding of the variables in the data base. An exploratory analysis was done on the database using frequency distributions to check for missing fields, omissions, entry errors, double entries to further ascertain the suitability of the database. Analysis was based on the research questions, research hypothesis and the operationalization of the study variables. Analysis started with a descriptive statistic of the variables which included measures of central tendencies for continuous variables, bar and pie charts for nominal variables.

RQ1 was about the relationship between the consumption of fresh (raw) produce and the occurrence of salmonellosis. These are two nominal (dichotomous) variables. The Pearson Chi Square statistic is commonly used for testing relationships between categorical variables. This test was used to test the hypothesis that there is no relationship between the consumption of fresh (raw) produce and the occurrence of salmonellosis.

RQ2 was about the relationship between the demographic characteristics (age, sex, race, ethnicity, and county of residence) of Maryland population and the occurrence of contracting salmonellosis through the consumption of fresh (raw) produce. Occurrence of salmonellosis is a dichotomous variable. Therefore, RQ2 was analyzed using logistic regression. Logistic regression is a type of regression wherein the outcome variable is categorical (as in the case of occurrence of salmonellosis after eating fresh produce in the question) and the independent (or predictor) variables are continuous or categorical. In a situation where the prediction is on an outcome categorical variable that has only two parameters, binary logistic regression is used in modeling a response for the dependent variable using the independent variables. In this study, binary logistic regression afforded me the opportunity to check for the odds ratio for developing salmonellosis from the independent variables as well as their relationships with the dependent variable.

RQ3 was about relationships between salmonellosis foodborne outbreak from fresh produce and seasonal patterns in Maryland. This is also comparing two categorical variables based on their operationalizations. Standard logistic regression entry method was used for the independent variables. Logistic regression was used to assess the potential effects of confounding variables. Likewise, this question was analyzed using Pearson's Chi-Squared test.

RQ4 was about whether the consumption of fresh (raw) produce and the development of salmonellosis is modified by geographic location. This question was analyzed using multivariable logistic regression which include a product term between differing geographic locations and consumption of raw produce.

RQ5 was about whether the relationship between salmonellosis outbreaks and the consumption of fresh produce is modified by the type of produce consumed (i.e. transmitting agent). This question was analyzed using multivariable logistic regression, with a product term included.

Threats to Validity

Threats to External Validity

A threat to external validity is usually a major obstacle to the generalization of study findings. This may arise due to sampling techniques, instruments used for data collection and insufficient sample size. This study used secondary data analysis that employed a standardized instrument for data collection. This is because according to Leedy and Ormrod (2013), a reliable instrument expresses the actual scores of the items evaluated and thus enhances and strengthens the study to provide stable and consistent results. There was no sampling of the data, rather, a census of all the available entries was used to ensure full inclusion of all the population elements thus ensuring generalizability of study findings.

Threats to Internal Validity

Threats to internal validity can diminish the assurance in saying that an association exists between the independent and dependent variables when in fact, the relationship is caused by a variable extraneous to the research context (Bhattacherjee, n.d.). Therefore, internal validity is relevant in studies that try to establish a causal relationship. Since this study was a secondary data analysis, much of the consideration for internal validity was based on how the parent study was conducted. Possible compromises to internal validity included measurement errors that could have arisen from varied sources, e.g., measuring the wrong variables, double data entry, lack of uniformity in study setting, different study instrument, dissimilar coding and varied interpretation of the measuring instrument by people. The methodology and evaluation of the parent study has been stated, noting standardization in the conduct of the study. Threats to internal validity may occur during data analysis and interpretations. This was averted by using the appropriate statistical methods to answer the research hypothesis.

Ethical Concerns

Not every research requires the collection of fresh data from participants. There is a large amount of existing data that can be used to generate new hypothesis or answer research questions (Tripathy, 2013). In order to guarantee that this study follows established ethical guidelines, I submited the study proposal to Walden University's Institutional Review Board (IRB) for review and approval. Approval number is 02-23-21-0426030. I wrote for the permission to use the data set of the FoodNet program from the MDH. I was granted access to the data set (see Appendix A). Since I worked with secondary data, the consent of the study subjects was presumed. However, in order to maintain confidentiality, and based on the conditions for use, all the data are anonymous as all traceable identifiers had been removed. I kept the database in my computer system with a password access ensuring that only the investigator had access to the data. Also, my data analysis in no way brought about any information that was able to re-identify the participants. The data set will be kept with me for 5 years after which it will be deleted.

Summary

Secondary data analysis was used for this study. A description of the source, personnel involved in the data collection, the methods employed, and standardization when the data was collected was described. The final evaluation is that the archival data sourced was suitable and adequate for answering the research questions for this study. Data analysis involved logistic regression and chi- squared analysis. The continuous variables were summarized by the use of the measures of central tendencies, while categorical data were described by the use of charts and graphs. Threats to both internal and external validity were addressed. Also, the ethical consideration for the study was stated. Analysis was done using SPSS version 27. Chapter 4 includes findings from the study.

Chapter 4: Results

Introduction

The purpose of this quantitative cross-sectional study was to investigate major risk factors involved with salmonellosis outbreaks in Maryland, methodically evaluate their influence on consumption of raw produce (predictor variable) and determine relationships between consumption of produce and the occurrence of foodborne salmonellosis (dependent variable). Types of contaminated produce associated with salmonellosis outbreaks included berries, cantaloupe, herbs, lettuce, spinach, sprouts, tomatoes, and watermelon. I investigated five associations between independent and dependent variables as stated in the research questions and hypotheses:

RQ1: Is there an association between the consumption of fresh produce and the occurrence of salmonellosis?

$H_0 1$: There is no association between the consumption of fresh produce and the occurrence of salmonellosis.

$H_a 1$: There is an association between the consumption of fresh produce and the occurrence of salmonellosis.

RQ2: Is there an association between demographic characteristics (age, sex, race and ethnicity) of Maryland and the risk of contracting salmonellosis through the consumption of fresh produce?

$H_0 2$: There is no association between demographic characteristics of Maryland and the risk of contracting salmonellosis through the consumption of fresh produce.

H_a2: There is an association between demographic characteristics of Maryland and the risk of contracting salmonellosis through the consumption of fresh produce.

RQ3: Is there an association between salmonellosis foodborne outbreaks from fresh produce and seasonal patterns in Maryland?

$H_0$3: There is no association between salmonellosis foodborne outbreaks from fresh produce and seasonal patterns in Maryland.

H_a3: There is an association between salmonellosis foodborne outbreaks from fresh produce and seasonal patterns in Maryland.

RQ4: Is the association between consumption of raw produce and development of salmonellosis modified by geographic location in Maryland?

$H_0$4: The association between consumption of raw produce and development of salmonellosis is not modified by geographic location in Maryland.

H_a4: The association between consumption of raw produce and development of salmonellosis is modified by geographic location in Maryland.

RQ5: Is the association between salmonellosis outbreaks and fresh produce consumption modified by the type of produce consumed?

$H_0$5: The association between salmonellosis outbreaks and consumption of fresh produce is not modified by the type of produce consumed.

H_a5: The association between salmonellosis outbreaks and consumption of fresh produce is modified by type of produce consumed.

This chapter provides details regarding findings and associated results . I also explain procedures for data preparation and use for secondary data analysis, the different statistical analyses used in addressing RQs, and a summary of results.

Exploratory Analysis of Secondary Data

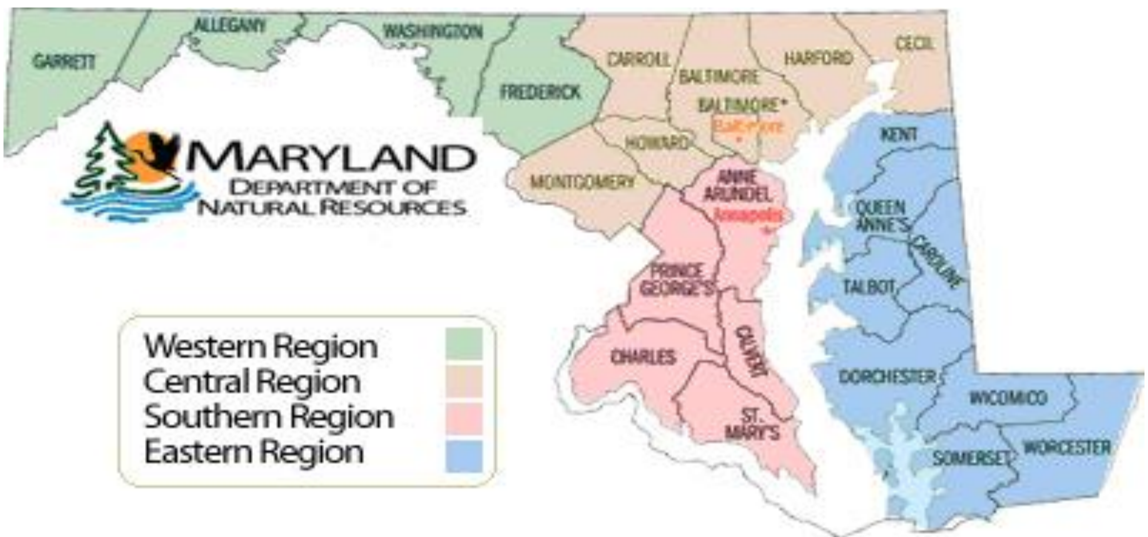
Archival data used for this study were obtained from the epidemiological unit of the MDH using the FoodNet program. It includes Maryland *Salmonella* surveillance data collected using a standard survey instrument (Gastroenteritis Case Report Form) developed by the CDC (see Appendix B). Data were collected via surveillance of laboratory-confirmed human *Salmonella* isolates. Identified *Salmonella* isolates were submitted to public health laboratories of the MDH for further confirmation and serotyping. Unusual serotypes were further sent to the CDC reference laboratory for further confirmation and characterization, and results were reported back to the MDH.

All data were published between 2010 and 2018. This data had been deidentified, and all information that could lead to conflicts involving confidentiality was removed. Some variables had to be recoded or transformed to fit needed variable operationalization. For example, the specific sites of contamination (named locations, institutions, or restaurants) were expunged from data but counties in Maryland where data were obtained were readily available. These counties were categorized into different Maryland regions for the purpose of analysis (see Figure 6). To address seasonal variations dates of data collection were coded into the following categories: December, January, February (winter) March, April, May (spring), June, July, August (summer), and

September, October and November (fall). A total of 8,352 entries were available for analysis.

Figure 6

Geographical Regions of Maryland



Results

Demographic Characteristics of Study Units

Table 2 includes demographics of study units included in the analysis. Most of the entries were Whites (61.5%), non-Hispanics (90.8%) and from the Central Region of Maryland (54.3%). Females were more than half of the population.

Table 2

Demographic Characteristics of Study Participants

Demographic characteristics	Frequency	Percentage
Age		
<5	1633	19.8
5-9	653	7.9

10-19	864	10.5
20-64	4010	48.5
65+	1103	13.3
Sex		
Female	4522	54.2
Male	3817	45.8
Race		
Asian	367	5.1
Black	2361	32.6
White	4457	61.5
Others*	68	0.9
Ethnicity		
Hispanic	679	9.2
Non-Hispanic	6685	90.8
Seasonal variation		
Winter	1167	14.1
Spring	1532	18.5
Summer	3331	40.2
Fall	2254	27.2
Regions		
Western	585	7.0
Central	4538	54.3
Southern	2158	25.8
Eastern	1071	12.8

*American Indian/Alaskan Native, Multi-racial, Pacific

The overall prevalence of salmonellosis by a positive culture can be seen across the age groups, with 5-9 being the least and 20-64, the highest. Table 3 is the prevalence of salmonellosis with respect to the demographics of the study units.

Table 3

Prevalence of Salmonellosis According to Demographic Characteristics

Demographic Characteristics	Occurrence of Salmonellosis*		χ^2	p-value
	Yes	No		
Age				
<5	1614(19.8)	19(15.6)	4.362	0.359
5-9	646(7.9)	7(5.7)		
10-19	853(10.5)	11(9.0)		
20-64	3947(48.5)	63(51.6)		
65+	1081(13.3)	22(18.0)		
Total	8141(100)	122(100)		
Sex				
Male	4451(54.2)	71(56.8)	0.338	0.561
Female	3763(45.2)	54(43.2)		
Total	8214(100)	125(100)		
Race				
Asian	362(5.1)	5(4.4)	4.425	0.219
Black	2332(32.7)	29(25.4)		
White	4377(61.3)	80(70.2)		
Others	68(1.0)	0(0.0)		
Total	7139(100)	114(100)		
Ethnicity				
Hispanic	665(9.2)	14(11.9)	1.001	0.317
Non-Hispanic	6581(90.8)	104(88.1)		
Total	7246(100)	118(100)		

*For Occurrence, data are reported as number (percentage of population).

The race category that falls under the group “other’ comprises the American Indians/Alaskan Native, Multi-racial, and Pacific Islanders has as small as 1% prevalence of salmonellosis in Maryland. However, none of the groups was statistically significant ($p > 0.05$). Table 4 is the list of all the counties involved in the survey and the frequency of participants examined per county. The Table shows that Baltimore City had the highest frequency of 15.9% while Garrett County had the lowest frequency with 0.2% of the total population.

Table 4

Frequency Distribution of Participants per County

Counties	Frequency	Percentage
Allegany	65	.8
Anne Arundel	854	10.2
Baltimore City	1325	15.9
Baltimore County	1016	12.2
Calvert	120	1.4
Caroline	86	1.0
Carroll	206	2.5
Cecil	115	1.4
Charles	169	2.0
Dorchester	111	1.3
Frederick	354	4.2
Garrett	18	.2
Harford	282	3.4
Howard	357	4.3
Kent	56	0.7
Montgomery	1237	14.8
Prince Georges	867	10.4
Queen Annes	111	1.3
Somerset	109	1.3
St. Mary's	148	1.8
Talbot	99	1.2
Washington	148	1.8
Wicomico	323	3.9
Worcester	176	2.1
Total	8352	100.0

The various types of contaminated produce associated with salmonellosis outbreaks identified in the secondary data were berries, cantaloupe, herbs, lettuce, spinach, sprouts, tomatoes, and watermelon. Table 5 details the history of either eating or not eating these raw produces.

Table 5

History of Consuming Raw Produce Among Study Participants

Raw produce	Frequency	Percentage
Cantaloupe (n=3099)		
Yes	573	18.5
No	2526	81.5
Spinach (n=3066)		
Yes	553	18.0
No	2513	82.0
Sprouts (n=3088)		
Yes	83	2.7
No	3005	97.3
Watermelon (n=3089)		
Yes	818	26.5
No	2271	73.5
Tomatoes(n=3089)		
Yes	1344	43.5
No	1745	56.5
Berries (n=3070)		
Yes	998	32.5
No	2072	67.5
Lettuce (n=3114)		
Yes	1519	48.8
No	1595	51.2
Herbs (n=3046)		
Yes	401	13.2
No	2645	86.8

Overall, 75.3% of all the participants consume raw produce. Table 6 is the prevalence of salmonellosis according to the different types of raw produce consumed by the surveyed population. As seen in the Table, the prevalence of salmonellosis averaged 97% for all the raw produce.

Table 6

Prevalence of Salmonellosis According to the Different Types of Raw Produce Consumed

Types of raw produce consumed	Occurrence of *Salmonellosis**	
	Yes	No
Cantaloupe	558 (9.1)	15 (7.9)
Spinach	536 (8.8)	17 (9.1)
Sprout	80 (1.3)	3 (1.6)
Watermelon	797(13.1)	21(11.1)
Tomatoes	1298 (21.3)	46 (24.3)
Berries	966 (15.8)	32 (16.9)
Lettuce	1478 (24.2)	41(21.7)
Herbs	387 (6.3)	14 (7.4)
Total	6100(100)	189(100)

*For Occurrence, data are reported as number (percentage of population).

RQ1

This question addressed the association between the consumption of fresh (raw) produce and the occurrence of salmonellosis. These are two nominal (dichotomous) variables and Pearson Chi Square statistic was used to test the relationships between these categorical variables. Tables 7 and 8 show the result of the analysis. Table 7 compared the consumption of each raw produce with the occurrence of salmonellosis while in Table 8, consumption of raw produce was taken as a whole to see its relationship with the occurrence of salmonellosis. From Table 7, not consuming tomatoes was statistically significant compared to the consumption of tomatoes in the occurrence of salmonellosis, χ^2 (1) = 6.54, (p = 0.011). However, in Table 8, there was no statistically significant relationship between the consumption of raw produce and the occurrence of salmonellosis χ^2 (1) = 2.56, (p > 0.05).

Table 7

Association between Type of Raw Produce Consumed and Occurrence of Salmonellosis

Type of raw produce	Occurrence of *Salmonellosis**		χ^2	p-value
	Yes	No		
Cantaloupe				
Yes	558 (97.4)	15(2.6)	0.000	0.995
No	2460 (97.4)	66(2.6)		
Spinach				
Yes	536 (96.9)	17(3.1)	0.414	0.520
No	2448 (97.4)	65(2.6)		
Sprouts				
Yes	80 (96.4)	3(3.6)	0.303	0.582
No	2929 (97.4)	79(2.6)		
Watermelon				
Yes	797 (97.4)	21(2.6)	0.000	0.984
No	2213 (97.4)	58(2.6)		
Tomatoes				
Yes	1298 (96.6)	46(3.4)	6.540	**0.011**
No	1711 (98.1)	34(1.9)		
Berries				
Yes	966 (96.8)	32(3.2)	1.631	0.202
No	2022 (97.6)	50(2.4)		
Lettuce				

Yes	1478 (97.3)	41(2.7)	0.050	0.823
No	1554 (97.4)	41(2.6)		
Herbs				
Yes	387(96.5)	14(3.5)	1.126	0.289
No	2577(97.4)	68(2.6)		

*For Occurrence, data are reported as number (percentage of population).

Table 8

Association Between Consumption of Raw Produce and Occurrence of Salmonellosis

Consumption of raw produce	Occurrence of *Salmonellosis** Yes	No	χ^2	p-value
Yes	2270(97.1)	68(2.9)	2.56	0.110
No	877(98.1)	17(1.9)		

*For Occurrence, data are reported as number (percentage of population).

RQ2

RQ2 assessed the risk of contracting salmonellosis through the consumption of fresh (raw) produce and the demographic characteristics (age, sex, race and ethnicity) of the studied Maryland population. Multiple logistic regression was used for this analysis. Table 9 shows a preliminary analysis of the association of the variables, while Table 10 shows the result of the logistic regression analysis. From Table 10, it can be seen that Ethnicity with Hispanic subset is the only demographic factor that is statistically significant in predicting the risk of contracting salmonellosis from eating fresh produce ($p < 0.05$). It will also be observed from the Table that the demographic factor race was not captured as Table 9 showed that one of the cells for others (under race) was empty and cannot be used for the analysis.

Table 9

Association Between Demographic Characteristics and the Risk of Contracting Salmonellosis

Demographic characteristics	Contract salmonellosis*		χ^2	p-value
	Yes	No		
Age				
<5	306(97.1)	9(2.9)	1.751	0.781
5-9	189(96.9)	6(3.1)		
10-19	230(98.3)	4(1.7)		
20-64	1183(97.1)	35(2.9)		
65+	354(96.5)	13(3.5)		
Sex				
Male	1322(97.0)	39(2.9)	0.021	0.884
Female	948(97.0)	29(3.0)		
Race				
Asian	88(98.9)	1(1.1)	1.833	0.608
Black	539(96.9)	17(3.1)		
White	1483(96.9)	47(3.1)		
Others	23(100.0)	0(0.0)		
Ethnicity				
Hispanic	225(94.9)	12(5.1)	3.932	0.047
Non-Hispanic	1983(97.3)	56(2.7)		

*For Occurrence, data are reported as number (percentage of population).

However, when the analysis was conducted with those who consumed raw produce (see Table 9), it was found that the residents of Maryland who were non-Hispanic had a higher chance of contracting salmonellosis compared to their Hispanic counterparts, χ^2 (1) = 3.93, (p = 0.047). Similarly, a multiple logistic regression (see Table 10) confirmed this finding by showing that residents of Maryland who were of Hispanic ethnicity had significantly less odds (about 47.5%) of contracting salmonellosis compared to the non-Hispanics via the consumption of the raw produce (p = 0.028).

Table 10

Multiple Logistic Regression Analysis Showing the Risk of Contracting Salmonellosis by Demographic Characteristics of Study Participants

Demographic characteristics	Odds Ratio (OR)	95% C. I. of OR		p-value
		Lower limit	Upper limit	
Age group				
<5	1.484	0.614	3.586	0.381
5-9	1.430	0.521	3.923	0.488
10-19	2.458	0.780	7.742	0.124
20-64	1.324	0.690	2.544	0.399
65+	1.0			
Sex				
Male	1.073	0.655	1.759	0.780
Female	1.0			
Ethnicity				
Hispanic	0.475	0.245	.921	0.028
Non-Hispanic	1.0			

RQ3

This research question addressed the associations between salmonellosis foodborne outbreak from fresh produce and seasonal patterns in Maryland. A logistic regression analysis was used for this analysis, Table 12 shows that Summer and Spring are significant at p = 0.001. Figure 7 shows that the highest foodborne outbreak occurs during Summer of the years studied. However, in the analysis to check the association between salmonellosis foodborne outbreak from fresh produce (among those who consume fresh produce) and seasonal variation, Summer (46.9%) had the highest recorded occurrence of salmonellosis foodborne outbreak from fresh produce. Table 11 shows that chi-square analysis indicates that seasonality is significantly associated with salmonellosis.

Table 11

Association Between Salmonellosis Foodborne Outbreak and Seasonal Patterns

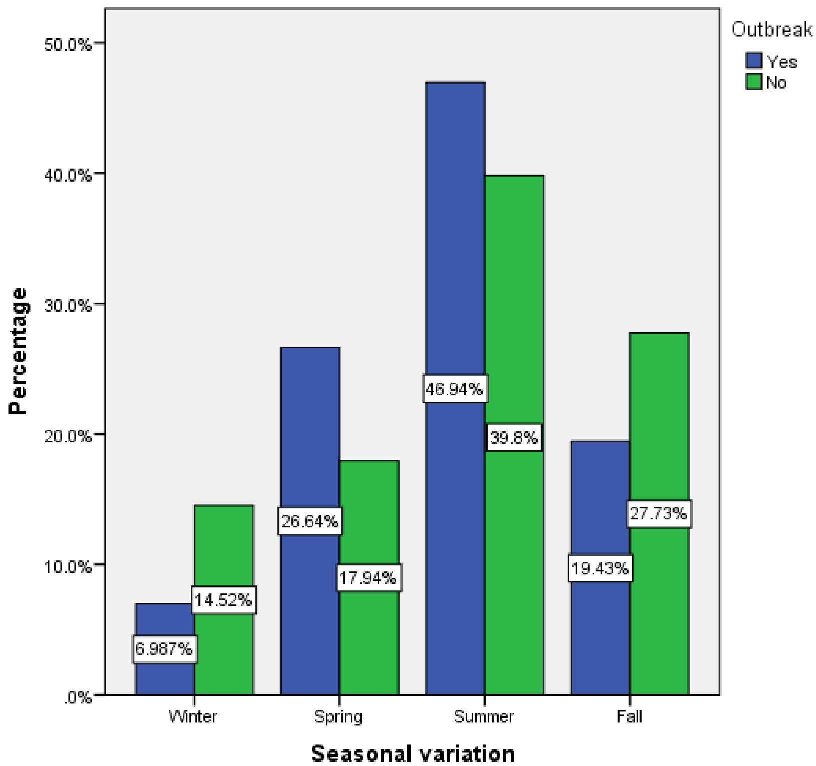
Seasonal patterns	Salmonellosis foodborne outbreak*		χ^2	p-value
	Yes	No		
Winter	32(7.0)	1098(14.5)	20.25	0.000
Spring	122(26.6)	1357(17.9)	22.22	0.000
Summer	215(46.9)	3010(39.8)	9.58	0.000
Fall	89(19.4)	2097(27.7)	15.14	0.001
All Seasons	458(5.7)	7562(94.3)	51.471	0.001

*For Occurrence, data are reported as number (percentage of population).

The chi square for Seasonality (grouped seasons) and outbreak of salmonellosis, Summer had the highest percentage, followed by Spring for the years studied.

Figure 7

Seasonal Variation of Salmonellosis Foodborne Outbreak from Fresh Produce



Likewise, the multinominal logistic regression also confirmed Spring and Summer as the seasons with the highest odds of *Salmonella* foodborne outbreak ($p < 0.05$) (Table 12).

Table 12

Logistic Regression Analysis of Salmonellosis Foodborne Outbreak from the Consumption of Raw Produce and Seasonal Patterns

Seasonal Patterns	Odds Ratio	95% C. I. of OR		p-value
		Lower limit	Upper limit	
Winter	0.687	0.455	1.035	0.073
Spring	2.118	1.598	2.807	**0.001**
Summer	1.683	1.683	2.168	**0.001**

Fall	1.0

RQ4

RQ4 addressed whether the consumption of fresh produce and the development of salmonellosis is modified by geographic location. The question was analyzed using Chi square statistics and logistic regression. The logistic regression analysis was to check the odds of predicting the development of salmonellosis by geographical location for the consumption of fresh produce. Tables 13 and 14 show the results from the Chi-Squared analysis and the logistic regression. Table 13 shows that all regions had a significant association with the occurrence of salmonellosis and the Eastern region had the highest percentage (99%) salmonellosis cases. An analysis for effect modification for the joint effects of consumption of raw produce and the specific geographic locations on the odds of salmonellosis also showed that the addition of the Eastern location increased the odds for the occurrence of salmonellosis (see Table 15). This is seen in Model 4 of the analysis. The other locations did not have any appreciable effect in the odds of developing salmonellosis. While the ORs were not derived from the interaction terms, the enter method was used where all explanatory variables were selected in block as a single step and applied to the model.

Table 13

Association Between Development of Salmonellosis and Geographical Regions

Regions	Salmonellosis foodborne outbreak*		χ^2	p-value
	Yes	No		
Western	144(92.3)	12(7.7)	16.93	0.000
Central	1156(98.0)	24(2.0)	13.05	0.001
Southern	680(95.9)	29(4.1)	56.59	0.000
Eastern	290(99.0)	3(1.0)	2.39	0.003
All Regions	2270(97.1)	68(2.9)	23.030	0.001

*For Occurrence, data are reported as number (percentage of population).

The Chi-Square analysis for Regions (“regionality”, grouped regions) and salmonellosis outbreaks is a comparison of Eastern region with other regions, which shows associations between other categorical variables but cannot provide either inferences nor causation.

Table 14

Logistic Regression Analysis of the Odds of Salmonellosis Foodborne Outbreak by Geographical Regions

Regions	Odds Ratio	95% C. I. of OR		p-value
		Lower limit	Upper limit	
Western	0.518	0.205	1.312	0.165
Central	1.031	0.629	1.691	0.904
Southern	0.983	0.579	1.668	0.949
Eastern	1.0			

The model in Table 16 was obtained by stepwise iteration of regression model from one or more explanatory variable. The effect of interaction of raw produce consumption per geographical regions is not statistically significant, except with the prediction of Eastern region as was also depicted by Tables 13 and 14.

Table 15

Joint Effect of Raw Produce Consumption and Geographic Location on the Development of Salmonellosis

Geographic location	Model 1 OR (95% C.I.)	Model 2 OR (95% C.I.)	Model 3 OR (95% C.I.)	Model 4 OR (95% C.I.)
Western	0.68 (0.49-0.94)	0.58 (0.41-0.82)	0.70 (0.46-1.04)	2.90 (0.32-26.54)
Central		0.76 (0.62-0.94)	0.92 (0.69-1.23)	3.85 (0.43-34.53)
Southern			1.34 (0.96-1.88)	5.60 (0.62-50.65)
Eastern				4.23 (0.47-38.49)

Table 16

Interaction Effect Between Consumption of Raw Produce and Geographic Region

Variables in Equation	B	S.E.	Wald	df	Sig.	Exp(B)
Consumption of Raw Produce	-.491	.874	.316	1	.574	.612
Geographic Region	-.276	.450	.377	1	.539	.759
Consumption of Raw Produce by Geographic Region	.024	.353	.005	1	.945	1.025
Constant	-2.40	1.11	4.690	1	.030	.090

RQ5

Research question five examined if the relationship between salmonellosis outbreaks and the consumption of fresh (raw) produce is modified by the type of produce consumed (i.e., transmitting agent). This question was analyzed using Chi Squared test to see the association between the type of produce consumed and salmonellosis outbreak. Logistic regression was also done to check the predictability of the type of produce consumed and development of salmonellosis foodborne outbreak. Table 17 is the result of the Chi squared analysis showing that the following fresh produce: Spinach, watermelon, berries, lettuce, and herbs were significantly associated with salmonellosis outbreaks. However, Table 18 shows that none of the fresh produce was statistically significant in predicting salmonella outbreak.

Table 17

Association Between Salmonellosis Outbreaks and Raw Produce Consumption by Type of Produce Consumed

Type of produce consumed	Occurrence of salmonellosis*		χ^2	p-value
	Yes	No		
Cantaloupe				
Yes	36(6.3)	537(93.7)	0.013	0.908
No	162(6.4)	2364(93.6)		
Spinach				
Yes	50(9.0)	503(91.0)	6.806	0.009
No	151(6.0)	2362(94.0)		
Sprouts				
Yes	7(8.4)	76(91.6)	0.519	0.471
No	194(6.5)	2811(93.5)		
Watermelon				
Yes	66(8.1)	752(91.9)	4.060	0.044
No	137(6.0)	2134(94.0)		
Tomatoes				

Yes	96(7.1)	1248(92.9)	1.755	0.185
No	104(6.0)	1641(94.0)		
Berries				
Yes	84(8.4)	914(91.6)	8.785	0.003
No	116(5.6)	1956(94.4)		
Lettuce				
Yes	117(7.7)	1402(92.3)	5.320	0.021
No	90(5.6)	1505(94.4)		
Herbs				
Yes	36(9.0)	365(91.0)	4.240	0.039
No	165(6.2)	2480(93.8)		

*For Occurrence, data are reported as number (percentage of population).

Table 18

Multiple Logistic Regression Analysis of Salmonellosis Outbreak and Type of Raw Produce Consumed

Type of raw produce consumed	Odds Ratio	95% C. I. of OR		p-value
		Lower limit	Upper limit	
Cantaloupe				
Yes	0.632	0.385	1.037	0.069
No	1.0			
Spinach				
Yes	1.311	0.868	1.979	0.198
No	1.0			
Sprouts				
Yes	0.940	0.362	2.441	0.898
No	1.0			
Watermelon				
Yes	1.347	0.899	2.019	0.149
No	1.0			
Tomatoes				
Yes	0.897	0.615	1.310	0.574
No	1.0			
Berries				
Yes	1.417	0.992	2.025	0.055
No	1.0			
Lettuce				
Yes	1.262	0.871	1.829	0.219
No	1.0			

Herbs				
Yes	1.444	0.925	2.253	0.106
No	1.0			

Furthermore, in Table 19 all the individual explanatory variables were added to the Product of the recoded variable. The Consumption of Raw Produce variable (>CRP*Produce Variable<) and the interaction effect between the consumption of raw produce and type of raw or fresh produce. It can be seen from Table 19 that a binary logistic model is obtained from the product of recoded variable added to each individual item in the explanatory variables, namely cantaloupe, spinach, sprouts, watermelon, tomatoes, lettuce, and herbs. This model shows tomatoes as the only statistically significant raw produce (0.005).

Table 19

Interaction Effect Between Consumption of Raw Produce and Type of Produce

Variables in Equation	B	S.E.	Wald	df	Sig.	Exp(B)
Consumption of Raw Produce (CRP)	1.872	1.818	1.061	1	.303	6.502
CRP by Cantaloupe	.048	.355	.018	1	.893	1.049
CRP by Spinach	.144	.336	.182	1	.669	1.154
CRP by Sprout	-.422	.625	.455	1	.500	.656
CRP by Watermelon	.144	.327	.192	1	.661	1.154
CRP by Tomato	-.888	.314	7.990	1	**.005**	.412
CRP by Berries	-.274	.280	.960	1	.327	.760
CRP by Lettuce	.384	.288	1.780	1	.182	1.468
CRP by Herbs	-.123	.346	.126	1	.723	.885

	Constant	-3.788	.695	29.74	1	.000	.023

Summary

In this chapter, I addressed how secondary data were prepared and cleaned for data analysis. Findings involving RQs were also addressed. A total of 8325 study units were included in data analysis. Most were Whites (61.5%) and non-Hispanics (90.8%) and from the central region of Maryland (54.3%). The average prevalence of salmonellosis in all the different demographic categories is 98%. However, none of the demographic characteristics had any significant association with the occurrence of salmonellosis.

Overall, 75.3% of all the participants consume raw produce. These includes cantaloupe, spinach, sprout, lettuce, berries, herbs, watermelon, and tomatoes. There was no statistically significant relationship between the consumption of raw produce and the occurrence of salmonellosis $\chi2$ (1) = 2.56, ($p > 0.05$). Nonetheless, residents of Maryland who were non-Hispanic had a higher chance of contracting salmonellosis compared to their Hispanic counterparts, $\chi2$ (1) = 3.93, ($p = 0.047$).

Also, the highest foodborne outbreak occurs during summer of the years studied. The Eastern region of Maryland had the highest association with occurrence of salmonellosis χ^2 (3) = 51.471, ($p = 0.001$). Spinach, watermelon, berries, lettuce, and herbs were significantly associated with salmonellosis outbreaks. However, none of the fresh produce studied was statistically significant in predicting salmonella outbreak. Chapter 5 discusses how *Salmonella* foodborne outbreaks from the consumption of fresh produce has impacted the food supply chain, limitation of the study, and recommendation on what can be done to prevent further outbreak. Also discussed in the next chapter, is the positive social change implication that this study is suggesting to both produce farmers, consumers, and the local health community educators on mechanism of transfer of *Salmonella* across the produce supply chain.

Chapter 5: Discussion, Conclusions, and Recommendations

Introduction

The CDC (2021) estimated about 1.35 million infections, 25,500 hospitalizations, and 420 deaths in the U.S. every year due to foodborne illnesses. The state of Maryland has been affected by preventable foodborne outbreaks. Therefore, this study is designed to evaluate outbreaks due to consumption of raw produce, demographic characteristics of the study population, raw produce consumption and seasonal variation, regional outbreaks, and agents of transmission, as well as occurrence of salmonellosis. Members of the *Salmonella* genus are identified as the most common enteric pathogen in the state with gram-negative and rod-shaped peritrichous flagella resulting in symptoms such as chills, headaches, nausea, and vomiting (FDA, 2012).

I used the system thinking model as the conceptual framework for this study. I specifically traced fresh produce from pre-harvest to final consumers throughout the farm-to-fork process. Modes of transmission, unique population characteristics and distribution, ethnicity, age groups, race, and seasonal variation were parameters used for evaluation. Irrigation water may not only be a source of *Salmonella* contamination of fresh produce and fruits, but a conduit for transmission (Liu et al., 2018). This may suggest why coastal communities in Maryland that depend on well water can be possible regions for salmonellosis spikes. To enhance control of agricultural water for use on produce, the FDA in 2013 introduced the Produce Safety Rule under the FSMA for prevention of waterborne outbreaks.

Interpretation of the Findings

RQ1

Using Pearson chi-square tests of relationships between categorical variables, I determined that consumption of tomatoes was not statistically significant in terms of occurrence of salmonellosis. This relationship is based on categorical differences between actual and observed responses in the chi-square analysis rather than percentages or proportions. Therefore, during hypothesis testing, there was no association between consumption of fresh produce and occurrence of salmonellosis. There was no statistically significant association between consumption of raw produce and occurrence of salmonellosis (χ^2 (1) = 2.56, $p > 0.05$).

Recent foodborne outbreaks involving fresh produce have implicated *Salmonella* as etiologic agents associated with population-based illnesses (Leang, 2013). The life cycle of tomatoes from pre-harvest to post-harvest especially when grown on a commercial scale production depends on some form of irrigation water and manure application for fertilization. Tomatoes were found to be directly linked to outbreaks of salmonellosis (van Dyk et al., 2016). Therefore, focusing on irrigation water during the pre-harvest stage and using contaminated water for the washing of fresh produce during the post-harvest stages could also lead to contamination. The scar spot on the tomato fruit is a possible site for *Salmonella* to thrive. Hanning et al. (2009) said dilated plant cells on the surface of tomatoes when exposed to irrigation water contaminated with *Salmonella* can absorb this pathogen through openings on the stem scar. *Salmonella* pathogens can internalize and proliferate in plants like tomatoes, but the mechanism of transfer does not prove active adhesion to fresh produce as observed in this study (Liu et al., 2018). However, poor sanitation practices at process facilities and personal hygiene of food service workers can be consequential sources of transmission of salmonellosis.

RQ2

A multiple logistic regression statistical analysis was used to evaluate demographic characteristics of the residents of Maryland who were under active surveillance for salmonellosis outbreaks between 2010 and 2018. Results of statistical analysis showed that, of all demographic factors, ethnicity is statistically significant in terms of predicting risks of contracting salmonellosis from eating fresh produce ($p < 0.05$); $\chi^2 (1) = 3.93$, ($p = 0.047$; see Table 9). Furthermore, when the ethnicity subset of the Maryland population (Hispanic and non-Hispanics) was analyzed using multiple logistic regression, results showed that Hispanic residents of Maryland had significantly lower odds for contracting salmonellosis compared to non-Hispanics due to consumption of raw produce (OR = 0.475, $p = 0.028$; see Table 10). Jiang et al. (2015), in a similar study reported that non-Hispanic Whites represented the majority of salmonellosis outbreak cases observed.

RQ3

Analysis of associations between salmonellosis foodborne outbreaks and seasonal variation was done using multinominal logistic regression analysis. Summer (46.9%) was the period during which recorded occurrence of salmonellosis foodborne outbreaks from fresh produce were most frequent and was significantly associated with salmonellosis occurrence; see (Figure 7 and Table 13). Spring had the next highest percentage of salmonellosis occurrence (26.6%) and was also statistically significantly associated with salmonellosis occurrence. The available secondary data set did not make provisions for cofounding variables such as temperature and precipitation. The possibility of extreme temperature and precipitation due to global warming could account for the variations in the salmonellosis outbreak numbers between fall and spring seasons.

Luo et al. (2015) said *Salmonella* peaks are seasonal, and outbreak levels correlated with increased temperature and rainfall ($p < 0.05$). According to Wilson (2015), salmonellosis is more associated with summer and spring seasons. Warm temperatures lead to frequent outdoor activities and occasions for people to eat out and spend longer hours outdoors.

RQ4

Chi square statistics analysis found that the Eastern region had the highest percentage of occurrences (99%, followed by the Central region at 98%), see Table 13. Also, all regions were significantly associated with occurrence of salmonellosis. This result is confirmed by a recent study conducted by Jiang et al. (2015), more intense temperatures extremes in coastal areas could result in more cases of salmonellosis by providing warmer niches conducive to greater bacteria amplification. When the surrounding temperature in the environment warms up, the water molecules in the coastal estuaries, streams or rivers evaporate and results in rains that lead to flooding. The geographical locations of the four regions in Maryland is a particularly unique variable because it defines both the extreme precipitation and flooding events in the state (Moser et al., 2012; Semenza et al, 2012). Also, the logistic regression analysis was to check the odds of predicting the development of salmonellosis by geographical location for the consumption of fresh produce. Table 14 shows the results from the chi-squared analysis and the logistic regression.

Applying the system thinking approach as should be throughout the study, it could be conceptualized that produce are transported across regions and food items could be contaminated in the process. Following the functional statewide food supply chain, pre-harvest practices and post-harvest measures, delivery and transportation, and food service at restaurants, schools or nursing homes are places where possible contamination can enter the supply chain.

RQ5

This variable was analyzed using the chi squared test to evaluate the association between the type of produce consumed and salmonellosis outbreak. From Table 15, the result of the chi squared analysis with respective p-value for the following fresh produce, namely, spinach, watermelon, berries, lettuce, and herbs were 0.009, 0.044, 0.003, 0.021, and 0.039, respectively. These p-values show a statistic that the listed fresh produce is significantly associated with salmonellosis outbreaks. However, the logistic regression model was also used to explore the test for any possible predictability of the type of produce consumed, and whether or not it could lead to the development of salmonellosis foodborne outbreak. It could be seen that Table 16 is a single regression model and none of the fresh produce is statistically significant in predicting salmonellosis outbreak.
The lack of significance in Table 16 is due to a blurring of the causal relationship resulting from people's eating patterns (eating more than one category of food, problems

with recall, reporting an outbreak but refusing to submit culture sample, and so on). According to Varga et al, (2020), while statistical analyses often uncover numerous associations, associations themselves do not convey predictive value. This inference has often time resulted in confusion between association and prediction, and has caused harm to clinicians, scientists, and ultimately, the patients. Similarly, in a search undertaken by NCBI PubMed with articles using search terms “diabetes” and “prediction” of all 1,910 abstracts evaluated, 39% (n = 745) reported metrics of predictive statistics, while 61% (n = 1,165) did not (Varga et al, 2020). Also, in another nationally representative samples of 188,819 and 164,113 children aged less than 5 years across India to study birthweight, it was also concluded that association does not imply prediction for the analysis of mortality and anthropometric failure (Swaminathan et al., 2020).

Salmonellosis outbreaks are traceable to contaminated produce, attributed to the mechanism by which *Salmonella* is attached or internalized into raw vegetables and fresh fruit (Hanning et al., 2009). Once attached to or internalized into fresh produce, *Salmonella* is transferred stepwise through the food supply chain in the farm-to-fork process. In a pulsed-field gel electrophoresis study, isolates of *Salmonella* from pond water were found to be genotypically identical to those in irrigation water (Hanning et al., 2009). Another study observed how *Salmonella* internalize underneath the stomata, which is considered helpful in the mechanism of transfer of *Salmonella*. *Salmonella* was observed in tissue underneath the stomata, making the morphology of the lettuce vulnerable for pathogen attachment (Kroupitski et al., 2011).

Limitations of the Study

Some national foodborne outbreaks of salmonellosis are under-reported in the data compiled by local health department covering cities, counties, and state health departments. The surveillance data are dynamic because data from previous years may vary as isolates are added or corrected (CDC, 2011). There are many missing cases in the Maryland FoodNet surveillance system. Not all persons with a *Salmonella* infection seek medical care; therefore, there is no way to account for the victims in this category in Maryland just like the national surveillance (Scallan et al., 2006). It can also be observed from Table 9 that the demographic factor race was not captured as Table 9 showed that one of the cells for “others” (under Race) was empty and cannot be used for the analysis. The laboratory identification of *Salmonella* isolates, their variable sources, and origin can pose a possible challenge or barrier to this study. Once a particular foodborne salmonellosis outbreak is suspected, the stool samples from affected persons are transferred to the diagnostic laboratories of the U.S. Department of Agriculture, Food Safety and Inspection Service, Animal and Plant Health Inspection Services, and National Veterinary Services laboratories for proper serotyping (CDC, 2011). My occupational affiliation with the Food Safety and Inspection Service in one of Maryland's counties did not influence the study’s outcome since the data was obtained as a secondary data from a credible; the Maryland Department of Health and Foodborne Disease Active Surveillance Network, the Maryland FoodNet surveillance system .

Recommendations

There should be a statewide mandate for all food service facilities (FSF) to adopt a common sanitizer for Facility-wide cleaning with a specific concentration or mixing ratio. This is important to eliminate FSF using discretionary choice of sanitizers, because some operators may not know how to use a test strip to determine effectiveness. The strength of a sanitizer should always be in the concentration of the sanitizer chemistry. An integrated HACCP plan should be introduced into the fresh produce food supply chain so that produce can be monitored for possible contamination and spoilage. Introduction of the system thinking approach would encourage the retention of purchase receipts for a specified period, to assist in pre-harvest and post-harvest tracking. In the event of recalls, it could aid in conducting an effective traceability investigation. Appendix C contains some tips for *Salmonella* prevention.

Implications for Positive Social Change

One positive social change implication is to provide both produce farmers, consumers, and local community health educators the mechanism of transfer of *Salmonella* through the produce supply chain so farmers can help prevent contamination of produce from the preharvest to postharvest stages of production. Also, the knowledge gained from this study will help reduce the burden of food-borne disease and increase awareness of issues related to the food supply chain.

The local food safety policy makers can integrate the system thinking model into more functional food prevention guidelines to improve food safety from farm-to-fork for Marylanders. These results support the introduction of new eco-friendly produce sanitizers, with specific mixing ratios and test strips, for mandatory use by all food service facility FSF. This eco-friendly sanitizer will eliminate the use of “sanitizer tablet” purchased over the counter by some licensed FSF, who buy these tablets and dissolve them in water to use in their 3-compartment sinks during manual dishwashing. I have personally seen these during my FSF inspection, and they do not meet the food code (COMAR 10.15.03.26). Including an eco-friendly sanitizer into the supply chain at the postharvest washing stage before transportation, followed by the introduction of the HACCP plan into produce after delivery to restaurant or consumption outlets will enhance produce safety for all Marylanders.

According to Hanning (2009), several produce items specifically have been identified in outbreaks, and the ability of *Salmonella* to attach or internalize into vegetables and fruits may be factors that make these produce items more likely to be sources of *Salmonella*. The foodborne *Salmonella* is also traceable to zoonotic sources, i.e., from the intestinal tracks of animals including reptiles, amphibians, live poultry chickens, ducklings, geese, and turkeys that may pass them out in their feces to the environment resulting in contaminated run-off and non-point source pollution (Behravesh et al., 2014). Splashes from feces (manure), irrigation water, or surface run-off adhere to the produce resulting in the contamination of the produce either at the preharvest or post-harvest stages. Greater efforts should be made to ensure that irrigation and wash water is of acceptable water quality. Potable quality may not be possible or feasible but meeting the full body contact standard would be a reasonable alternative.

Conclusion

The frequent presence of *Salmonella* outbreaks in the food supply chain despite significant advances in sanitation chemistry, provision of potable water, HACCP plan, and highly regulated surveillance procedures is of concern (Sánchez-VargasaMaisam et al., 2011). This ubiquitous Gram-negative enterobacteria capable of causing harm to humans should be greatly reduced in the food supply to prevent serious and sometimes fatal illness in children below 5 years of age, adults older than 65 years, and the immune compromised individuals.

This study was specifically designed to use system thinking to navigate the food supply chain for fresh produce from preharvest through postharvest to end consumers, and it points to possible sources of contamination. This study clarifies the association between (a) the consumption of raw (fresh) produce and the occurrence of salmonellosis, (b) the demographic characteristics of the study population and the occurrence of salmonellosis, (c) raw (fresh) produce consumption and seasonal variation in the occurrence of salmonellosis, (d) site of outbreak and the occurrence of salmonellosis, and (e) agent (vehicle) of transmission and occurrence of salmonellosis.

It is interesting to note that there is an association between the consumption of fresh (raw) produce and the occurrence of salmonellosis. There is an association of the variables showing a statistical significance of demographic factors and salmonellosis outbreak, using the multiple logistic regression analysis. Hispanic as a subset is significantly associated with salmonellosis. Also, there is a confirmed statistical significance between summer and spring as the seasons with the highest odds of

Salmonella foodborne outbreak. The Eastern region of Maryland also had the highest percentage of occurrence of salmonellosis (99%), followed by the Central region with 98%. However, spinach, watermelon, berries, lettuce, and herbs have a statistically significant and associated with salmonellosis outbreaks.

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Appendix A: Data Use Agreement

DATA USE AGREEMENT

This Data Use Agreement, effective 01/28/21, is entered into by and between (Barisua Ikpe (Data Recipient) and Maryland Department of Health Infectious Disease Epidemiology and Outbreak Bureau (Data Provider). The purpose of this Agreement is to provide Data Recipient with access to a Limited Data Set (LDS) for use in research in accord with the HIPAA and FERPA Regulations.

1. Definitions. Unless otherwise specified in this Agreement, all capitalized terms used in this Agreement not otherwise defined have the meaning established for purposes of the "HIPAA Regulations" codified at Title 45 parts 160 through 164 of the United States Code of Federal Regulations, as amended from time to time.

2. Preparation of the LDS. Data Provider shall prepare and furnish to Data Recipient a LDS in accord with any applicable HIPAA or FERPA Regulations.

3. Data to be included in the LDS. **No direct identifiers such as names may be included in the Limited Data Set (LDS).** The researcher will not name the Data Provider in the doctoral study that is published in Proquest unless the Data Provider makes a written request for the researcher to do so. In preparing the LDS, Data Provider or designee shall include the **data fields specified as follows**, which are the minimum necessary to accomplish the research: (Salmonella outbreaks, collection timeline, impacted fresh produce, demographics, Maryland City/Counties).

4. Responsibilities of Data Recipient. Data Recipient agrees to:

a. Use or disclose the LDS only as permitted by this Agreement or as required by law;

b. Use appropriate safeguards to prevent use or disclosure of the LDS other than as permitted by this Agreement or required by law;

c. Report to Data Provider any use or disclosure of the LDS of which it becomes aware that is not permitted by this Agreement or required by law;

d. Require any of its subcontractors or agents that receive or have access to the LDS to agree to the same restrictions and conditions on the use and/or disclosure of the LDS that apply to Data Recipient under this Agreement; and

e. Not use the information in the LDS to identify or contact the individuals who are data subjects.

5. Permitted Uses and Disclosures of the LDS. Data Recipient may use and/or disclose the LDS for its research activities only.

6. Term and Termination.

a. Term. The term of this Agreement shall commence as of the Effective Date and shall continue for so long as Data Recipient retains the LDS, unless sooner terminated as set forth in this Agreement.

b. Termination by Data Recipient. Data Recipient may terminate this agreement at any time by notifying the Data Provider and returning or destroying the LDS.

c. Termination by Data Provider. Data Provider may terminate this agreement at any time by providing thirty (30) days prior written notice to Data Recipient.

d. For Breach. Data Provider shall provide written notice to Data Recipient within ten (10) days of any determination that Data Recipient has breached a material term of this Agreement. Data Provider shall afford Data Recipient an opportunity to cure said alleged material breach upon mutually agreeable terms. Failure to agree on mutually agreeable terms for cure within thirty (30) days shall be grounds for the immediate termination of this Agreement by Data Provider.

e. Effect of Termination. Sections 1, 4, 5, 6(e) and 7 of this Agreement shall survive any termination of this Agreement under subsections c or d.

7. Miscellaneous.

a. Change in Law. The parties agree to negotiate in good faith to amend this Agreement to comport with changes in federal law that materially alter either or both parties' obligations under this Agreement. Provided however, that if the parties are unable to agree to mutually acceptable amendment(s) by the compliance date of the change in applicable law or regulations, either Party may terminate this Agreement as provided in section 6.

b. Construction of Terms. The terms of this Agreement shall be construed to give effect to applicable federal interpretative guidance regarding the HIPAA Regulations.

c. No Third Party Beneficiaries. Nothing in this Agreement shall confer upon any person other than the parties and their respective successors or assigns, any rights, remedies, obligations, or liabilities whatsoever.

d. Counterparts. This Agreement may be executed in one or more counterparts, each of which shall be deemed an original, but all of which together shall constitute one and the same instrument.

e. Headings. The headings and other captions in this Agreement are for convenience and reference only and shall not be used in interpreting, construing or enforcing any of the provisions of this Agreement.

IN WITNESS WHEREOF, each of the undersigned has caused this Agreement to be duly executed in its name and on its behalf.

DATA PROVIDER	**DATA RECIPIENT**
Signed: [signature]	Signed: [signature]
Print Name: Michelle Boyle	Print Name: Barisua Ikpe
Print Title: FoodNet Administrator	Print Title: Student/Reseacher

Appendix B: Survey Instrument Developed by the CDC

Gastroenteritis Case Report Form

Maryland Department of Health

Use this form for:	Complete Sections
☐ *Campylobacter*	**I and II**
☐ *Cryptosporidium*	I only
☐ *Salmonella* (non-*Typhi*)	**I and II**
☐ Shiga-toxin producing *E. coli*	**I and II**
☐ *Shigella*	I only
☐ *Yersinia*	I only
☐ Other:	I only

INSTRUCTIONS: Complete Section I for all pathogens and also Section II for only *Campylobacter, Salmonella*, and STEC cases. See **Interviewer Instructions** for more information. Submit completed forms to MDH FoodNet at fax #410-225-7615 or mdh.FoodNet@maryland.gov (*must be encrypted*).

SECTION I *(Complete for all pathogens)*

Investigation Data

INVESTIGATOR	INVESTIGATOR PHONE	NEDSS CASE ID#	INVESTIGATION ID# CAS
CASE REPORTED BY	LAB REPORT DATE	REPORT RECEIVED DATE	INTERVIEW DATE
CASE STATUS ☐ Confirmed ☐ Probable ☐ Suspect ☐ Unknown	CASE INVESTIGATED AS PART OF AN OUTBREAK?	☐ Yes ☐ No ☐ Unknown	OUTBREAK/CLUSTER ID
WORK OR SCHOOL RESTRICTIONS? ☐ Yes, *If yes, specify*: ☐ No		ADVISED OF PRECAUTIONS ☐ By phone ☐ Fact sheet	☐ In person ☐ In writing

Patient Data

LAST	FIRST	DATE OF BIRTH	AGE	SEX ☐ Male ☐ Female
STREET ADDRESS		HOMELESS ☐ No ☐ Yes		COUNTY
CITY	STATE / ZIP	TELEPHONE NUMBER(S)		
ETHNICITY ☐ Hispanic ☐ Not Hispanic ☐ Unknown	RACE (*Check all that apply)* ☐ Am. Indian/Alaskan Native ☐ Black/African American ☐ White ☐ Asian ☐ Other ☐ Unknown			
OCCUPATION, STUDENT, SITUATION	EMPLOYER, SCHOOL, DAYCARE			HIGH RISK ☐Healthcare ☐Food ☐ Daycare

Clinical Data

SYMPTOMS ☐ Asymptomatic	☐ Diarrhea ☐ Bloody diarrhea ☐ Fever (°F) ☐ Abdominal cramps	☐ Vomiting ☐ Nausea ☐ Chills ☐ Muscle aches	☐ Other: ☐ Other:
ONSET: DATE / TIME	DURATION ☐ still ill	OUTCOME ☐ Died, date: ☐ Survived ☐ Unknown	
PHYSICIAN VISIT ☐ No ☐ Yes →	PHYSICIAN NAME	PHYSICIAN PHONE #	STEC ONLY: HAVE HUS? ☐ No ☐ Yes
HOSPITALIZED ☐ No ☐ Yes →	ADMIT DATE / DISCHARGE DATE	HOSPITAL	ICU? ☐ No ☐ Yes
TRANSFERRED ☐ No ☐ Yes →	TRANSFER DATE / DISCHARGE DATE	TRANSFER HOSPITAL	
TREATED WITH ANTIBIOTICS ☐ No ☐ Yes ☐ Unknown →	*Name(s) of all antibiotics*:		

Laboratory Data ☐ ELR ☐ Epi-linked, no testing done

COLLECTION DATE	STATUS AT COLLECTION ☐ Hospitalized ☐ Outpatient ☐ Unknown	SPECIMEN TESTED ☐ Stool ☐ Blood ☐ Other: ☐ None	
Test Type ☐ Culture ☐ Unknown ☐ Non-culture, *specify:* (☐ EIA ☐ PCR ☐ Other)	LABORATORY NAME		ACCESSION #
AGENT IDENTIFIED	SEROTYPE	ISOLATE SENT TO STATE ☐ No ☐ Yes →	STATE ACCESSION #

Medical History

In the 30 days before illness, from			*to*	*, did [you/your child]:*
Medication Exposures	YES	NO	UNK	***If yes, specify name(s) or type(s):***
1. Take antacids or other medications to block acid?	☐	☐	☐	
2. Take any antibiotics?	☐	☐	☐	
3. Take any probiotics?	☐	☐	☐	
In the 6 months before illness, from ***to*** ***, were [you/your child]:***				
Comorbidities	YES	NO	UNK	
1. Diagnosed or treated for cancer?	☐	☐	☐	
2. Diagnosed or treated for diabetes?	☐	☐	☐	
3. Have abdominal surgery (e.g., removal of appendix or gallbladder, any stomach or intestinal surgery)?	☐	☐	☐	

Environmental Exposures

In the 7 days before illness, from ***to*** ***, did [you/your child]:***				
WATER-RELATED EXPOSURES	YES	NO	UNK	***If yes, details:***
1. Live in a home with a septic system?	☐	☐	☐	
2. Primarily use water from a well for drinking water?	☐	☐	☐	*Treatment:*
3. Drink any untreated water (pond, lake, river, etc.)?	☐	☐	☐	
4. Swim or wade in untreated water?	☐	☐	☐	*Where?*
5. Swim or wade in treated water (pool, hot tub, etc.)?	☐	☐	☐	*Where?*
ANIMAL CONTACT	YES	NO	UNK	
1. Have contact with an animal?	☐	☐	☐	
If yes, did [you/your child] have contact with a:				***If yes, details:***
a. Dog?	☐	☐	☐	
b. Cat?	☐	☐	☐	
c. Reptile or amphibian (frog, snake, turtle, etc.)?	☐	☐	☐	*Specify:*
d. Live poultry (chicken, turkey, hen, etc.)?	☐	☐	☐	
e. Pet bird (not live poultry)?	☐	☐	☐	
f. Cattle, goat, or sheep?	☐	☐	☐	*Specify:*
g. Pig?	☐	☐	☐	
h. Other animal?	☐	☐	☐	*Specify:*
i. Pet with diarrhea?	☐	☐	☐	
2. Visit, work, or live on a farm, ranch, or petting zoo?	☐	☐	☐	*Specify:*

Travel

In the 7 days before illness, from ***to*** ***, did [you/your child]:***	YES	NO	UNK
1. Travel to another state or country outside of your normal routine?	☐	☐	☐
In the 6 months before illness, from ***to*** ***, did [you/your child]:***	YES	NO	UNK
2. Travel to another country?	☐	☐	☐
3. Receive medical care in another country?	☐	☐	☐

List locations and travel dates:

a. Location:	From:	To:
b. Location:	From:	To:
c. Location:	From:	To:

Contacts

In the 7 days before illness, did [you/your child]:	YES	NO	UNK	*If yes, details:*
1. Have exposure to a daycare or nursery?	☐	☐	☐	*Name:*
2. Have a household or close contact with diarrhea?	☐	☐	☐	
In the 6 months before illness, did:	YES	NO	UNK	***If yes, what countries:***
1. Any member(s) of your household travel outside the U.S.?	☐	☐	☐	

[List all household contacts (ill or not ill), and any ill close contacts regardless of where they live (i.e., caregivers, boy/girlfriends, relatives, etc.). For all indicate if high risk; if symptomatic give onset and testing information.]

Name	Age	Relationship to Case	Symptoms		Onset Date	Lab Testing: Y/N, coll. date, result	High Risk		
			Yes	No			Day care	Health care	Food Svc.
			☐	☐			☐	☐	☐
			☐	☐			☐	☐	☐
			☐	☐			☐	☐	☐
			☐	☐			☐	☐	☐
			☐	☐			☐	☐	☐

Food Sources

In the 7 days before illness, from _______ to _______, did [you/your child]:				YES	NO	UNK
1. Attend any events where food was served? *(If yes, list below)*				☐	☐	☐
Event	Date	Location	Foods Eaten			
a.						
b.						
c.						
2. Eat at any restaurants? *(If yes, list below)*				☐	☐	☐
Name	Date	Location	Foods Eaten			
a.						
b.						
c.						
d.						

3. List all stores where food eaten prior to illness were purchased (e.g. grocery stores, ethnic markets, farm stands)

Name	Location	Shoppers Card Number
a.		
b.		
c.		
d.		

Complete Food History (next page) for ALL cases and Food Exposures (Section II) for ALL *Campylobacter*, non-*Typhi Salmonella*, and STEC cases.

Notes and Summary of Investigation

Food History *(For <u>all cases</u>, complete for the **7 days** before illness. If case was asymptomatic or the onset is unknown, complete for the **7 days** before collection. If the case is an infant or young child that is predominately breast-fed, formula-fed, or has limited food exposures, the following sections should also include responses from the individual who spends the MOST time with the case.)*

Date							
Morning / Breakfast							
Afternoon / Lunch							
Evening / Dinner							
Snacks / Other							

SECTION II

Food Exposures

[Instructions: Complete for all *Campylobacter*, non-*Typhi Salmonella*, and STEC cases. For all questions, ask for the 7 day period prior to onset of illness or, if unknown or asymptomatic, the 7 days prior to collection date. For questions answered YES, use the space on the right to provide additional details, such as the specific type of food and where food was purchased or eaten. If the case is an infant or young child that is predominately breast-fed, formula-fed, or has limited food exposures, the following sections should also include responses from the individual who spends the MOST time with the case.]

Respondent was: ☐ Self ☐ Parent ☐ Spouse ☐ Other (Specify):

		YES	NO	UNK	***If yes, details:***
1	Do you follow any special diet (e.g. vegan, kosher, gluten-free)?	☐	☐	☐	
2	Do you have any food allergies?	☐	☐	☐	
3	Do you take any dietary supplements, herbal supplements, or vitamins?	☐	☐	☐	

In the 7 days before illness, from to , did [you/your child] eat or drink any:

		YES	NO	UNK	***If yes, food details:***
1	Chicken or foods containing chicken?	☐	☐	☐	
If yes,	a. Chicken prepared outside the home?	☐	☐	☐	*Where?*
	b. Chicken at home?	☐	☐	☐	*Which part(s):*
	c. Ground chicken?	☐	☐	☐	
2	Turkey or foods containing turkey?	☐	☐	☐	
	a. Ground turkey?	☐	☐	☐	
3	Beef or foods containing beef?	☐	☐	☐	
If yes,	a. Beef prepared outside the home?	☐	☐	☐	*Where?*
	b. Ground beef?	☐	☐	☐	
If yes,	i. Undercooked or raw ground beef?	☐	☐	☐	
4	Any veal?	☐	☐	☐	
5	Pork or foods containing pork?	☐	☐	☐	
6	Lamb or mutton?	☐	☐	☐	
7	Liver (including pate)?	☐	☐	☐	
If yes,	a. Undercooked or raw liver?	☐	☐	☐	
10	Fish or fish products?	☐	☐	☐	
	a. Undercooked or raw fish (e.g., sushi)?	☐	☐	☐	
11	Seafood (e.g., crab, shrimp, oysters, clams)?	☐	☐	☐	*Specify:*
	a. Undercooked or raw seafood?	☐	☐	☐	*Which?*
12	Any other meat, poultry, or deli meats?	☐	☐	☐	*Specify:*
13	Frozen meals (e.g., pizza, soup, entrée)?	☐	☐	☐	*Specify:*
14	Dairy products (e.g., milk, yogurt, cheese, ice cream)?	☐	☐	☐	
If yes,	a. Pasteurized cow's or goat's milk?	☐	☐	☐	
	b. Unpasteurized milk or other dairy?	☐	☐	☐	*From where?*
	c. Soft cheese (e.g., queso fresco)?	☐	☐	☐	

Food Exposures (continued)

In the 7 days before illness, from ___ to ___, did [you/your child] eat or drink any:

	YES	NO	UNK	*If yes, food details:*
15 Eggs?	☐	☐	☐	
If yes, a. Eggs prepared outside the home?	☐	☐	☐	*Where?*
b. Eggs that were runny, raw, or uncooked foods made with raw eggs?	☐	☐	☐	*From where?*
16 Fresh cantaloupe?	☐	☐	☐	
17 Fresh watermelon?	☐	☐	☐	
18 Fresh (unfrozen) berries?	☐	☐	☐	*Specify:*
19 Unpasteurized, not from concentrate juice (sold at an orchard or farm, or commercially with label)?	☐	☐	☐	*From where?*
20 Fresh green onion or scallions?	☐	☐	☐	
21 Fresh cucumber?	☐	☐	☐	
22 Fresh, raw tomatoes?	☐	☐	☐	*Type(s):*
23 Fresh peppers (e.g., bell, hot, sweet)?	☐	☐	☐	*Specify:*
24 Fresh, raw lettuce?	☐	☐	☐	*Specify* loose (☐) *or* pre-packaged (☐)
25 Fresh (unfrozen), raw spinach?	☐	☐	☐	*Specify* loose (☐) *or* pre-packaged (☐)
26 Sprouts?	☐	☐	☐	*Specify:*
27 Other fresh fruits or vegetables eaten raw?	☐	☐	☐	*Specify:*
28 Fresh (not dried) herbs (e.g., basil, cilantro)?	☐	☐	☐	*Specify:*
29 Nuts or seeds?	☐	☐	☐	*Specify:*
30 Foods purchased online?	☐	☐	☐	
If yes, a. Grocery Delivery (Amazon Fresh, Peapod)?	☐	☐	☐	*Where?*
b. Meal Kit Delivery (Blue Apron, Meals on Wheels)?	☐	☐	☐	*Specify:*
31 Foods/baked goods sold out of someone's home?	☐	☐	☐	
32 Food from a food truck	☐	☐	☐	

[Click in box to type any additional notes.]

Appendix C: Five Facts for Salmonella Prevention

For Salmonella Prevention: Five Fast Facts and CDC's Tips

1. You can contract *Salmonella* from a variety of foods, including sprouts and other fruits vegetables, eggs, chicken, pork, processed foods, frozen pot pies, chicken nuggets, and stuffed chicken entrees. Contaminated foods usually look and smell normal.
2. *Salmonella* also can spread from animals to people and from people to people. Always wash your hands after contact with animals. Also wash your hands after using the toilet, changing diapers, or helping someone with diarrhea clean up after using the toilet. If you have a *Salmonella* infection, you should not prepare food or drinks for others until you no longer have diarrhea.
3. *Salmonella* illness is more common in the summer. Warmer weather and unrefrigerated foods create ideal conditions for *Salmonella* to grow. Be sure to refrigerate or freeze perishable foods, prepared foods, and leftovers within 2 hours (or 1 hour if the temperature outside is 90°F or hotter).
4. *Salmonella* illness can be serious and is more dangerous for certain people. Anyone can get a *Salmonella* infection, but some people are more likely to develop a serious illness, including children younger than 5, older adults, and people with immune systems weakened from a medical condition, such as diabetes, liver or kidney disease, and cancer or its treatment.
5. *Salmonella* causes far more illnesses than you might suspect. For every person with a *Salmonella* illness confirmed by a laboratory test, there are about 30 more people with *Salmonella* illnesses that are not reported. Most people who get food poisoning do not go to a doctor or submit a sample to a laboratory, so we never learn what germ made them sick. (CDC, 2019).