BASELINE OF *SALMONELLA* PREVALENCE IN RETAIL BEEF AND PRODUCE FROM HONDURAS AND MEXICO

by

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ABSTRACT

Salmonella is a common cause of food-borne illness worldwide, especially with must recent outbreaks. The sources of these outbreaks include contaminated animal products, including raw or undercooked meat as well as contaminated produce. The objective of the current study was 1) to develop a baseline for the prevalence and identification of *Salmonella* in beef and produce in Honduras, and produce in Mexico; and 2) to identify *Salmonella* serotypes in both Honduras and Mexico. A total of 393 retail whole muscle beef cuts samples and 383 produce samples (cantaloupes, cilantro, cucumbers, leafy greens, peppers, and tomatoes) were collected from major cities in different regions in Honduras in 4 different trips. A total of 514 produce samples were collected from major cities in Mexico in 6 different trips. Produce types and analysis followed the same as in Honduras. All retail beef and produce samples were tested using the *BAX® System PCR Assay* for *Salmonella* detection, with positive samples isolated for *Salmonella* using traditional cultural methods. Positive isolates were agglutinated and prepared for serotyping.

Overall, the prevalence of *Salmonella*-positive samples in Honduras (N = 393) retail beef resulted in 5.9% with a 95% CI [3.9, 8.7]. Whereas positive beef carcass swabs was 7.8% in both beef plants in Honduras (11/141). The most common serotypes identified in Honduras were *Salmonella* serotype Typhimurium followed by *Salmonella* serotype Derby. The overall prevalence of *Salmonella* in Honduras produce (N 383) was 2.4% with a 95% CI [1.2, 4.5]. Overall, the prevalence of *Salmonella*-positive samples in Mexico (N = 514) produce resulted in 2.1% with a 95% CI [1.2, 3.8]. The most common

serotype identified in Mexico was *Salmonella* serotype Meleagridis. Other serotypes found in Mexico were Typhimurium, Kentucky, and Newport.

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CHAPTER I

I. INTRODUCTION

Salmonella remains one of the world's leading causes of bacterial foodborne illness *(2, 3, 4, 8)*. It is estimated that *Salmonella* causes 93.8 million cases of gastroenteritis a year worldwide, with 155,000 deaths *(4)*. In the U.S., foodborne *Salmonella* remains a considerable challenge for the food industry, regulatory, government, and public health agencies, being the number one bacterial cause of death *(2, 4, 6)*. According to the Centers for Disease Control and Prevention (CDC), there are approximately 42,000 cases of salmonellosis reported annually in the U.S. as of 2010 *(2)*. Milder *Salmonella* infections are notably under-reported and/or under-diagnosed; therefore the actual number of infections may be significantly higher *(2)*. In addition, salmonellosis has a seasonal trend, with more cases reported in the summer than in the winter *(2)*.

According to the World Health Organization (WHO), more than 70% of acute diarrhea infections are caused by foodborne illness in Latin America *(9)*. Diarrhea is among the five main causes of death in children less than 5 years of age *(9)*. The World Health Organization estimates that 1.8 billion people die annually from diarrhea commonly acquired from food and water contaminated with enteric pathogens. Children in developing countries are the most vulnerable population group *(8)*.

In Latin America, one of the leading causes of bacterial acute gastroenteritis is *Salmonella*, similar to the U.S. *(7)*. Although developing countries have higher mortality rates from *Salmonella* infections, high morbidity rates are present in both industrialized and developing countries *(7)*. Despite advances in food sanitation and more active food chain surveillance, *Salmonella* infections result in a major economic burden to developing as well as industrialized countries. In regards to Latin America, *Salmonella* infections have a public health, economic and social impact much larger than industrialized countries such as the U.S. *(7).*

In 2007, Paniagua et al. conducted a study in Mexico City where 300 stool samples were collected from children, aged from 2 to 12 years old, presenting diarrhea symptoms. The control group matched for age, consisting of children (N = 80) showing no diarrhea symptoms. Children were tested for enteropathogens. Results indicated an estimated 52% of patients were infected with more than one enteropathogen. *Salmonella* was the second most common enteropathogen detected in this study. The high rate of enteropathogens detected in this study in Mexican children is a clear example on how diarrheal infections affect vulnerable populations (*5*). Similarly in Honduras, diarrhea infections are the second cause of morbidity and mortality in children under 5 years of age. During the years 2000 to 2004, there were an estimated 1,000 deaths caused by diarrhea infections *(1).*

Salmonella is an ubiquitous microorganism that has many different sources, such as food, animal and environmental sources *(2, 4, 8).* Foodborne transmission is recognized as the major cause of *Salmonella* infections, with many food sources and commodities implicated in these infections *(6).* In the U.S., *Salmonella* outbreaks are commonly associated with animal products such as beef; however, recent outbreaks have been linked to fruit and vegetables *(3)*. Cantaloupes, tomatoes, lettuce, and cilantro have been identified in previous outbreaks. Because *Salmonella* has the ability to attach or internalize in such produce, they are becoming a common source for *Salmonella.* Other environmental factors including contaminated water used for irrigation have also contributed to the increase of outbreaks linked to produce *(3)*.

Globalization has increased the demand for an expanded food industry production. Because of the outbreaks mentioned above, creating a baseline study for Latin America will help indicate the primary areas of concern and ultimately, allow for the control of foodborne *Salmonella.* Improving public health by protecting food depends on intensive cooperation by all countries, government institutions, private sector, academia and other organizations concerned with food safety.

Therefore there were two objectives to the current study. Objective 1) was to develop a baseline of the prevalence of *Salmonella* in produce and beef at retail markets and slaughter plants in Honduras and produce in Mexico. Objective 2) was to determine the serotypes of the *Salmonella* isolates commonly found in Mexico and Honduras.

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CHAPTER II

II. REVIEW OF LITERATURE

Honduras Agricultural Industry

Honduras is a small, mountainous Spanish-speaking country, located in Central America that relies heavily on agriculture *(5, 6)*. The agriculture economy employs about one-third of the population, however due to a largely mountainous terrain, most cultivable land is located on coastal plains, where plantations dominate *(5, 6)*. The dominant crops in Honduras are coffee and banana, although cantaloupes, pineapple, tobacco, cotton, African palm, and sugarcane are in abundance too *(5, 6)*. In addition cattle are the main source of livestock however due to the infrastructure and lack of pasture availability, there are limitations to both beef production and the dairy industry *(5, 6)*. Honduras principal trading partner for its beef exports is the U.S. with a 84.72% and then other Central American countries account for 8.8% *(59)*. Honduras exported nearly one million Kg of fresh and frozen beef to the U.S. in 2010 and in 2011, the beef exports increased to nearly 5 million Kg. For 2012 and 2013 this number is expected to triple.

In a report presented in May of 2011 by the U.S. Department of Agriculture (USDA) Foreign Agricultural Service (FAS), and the U.S. Agency for International Development (USAID), standards and regulations were able to assist Honduran meat processing plants recover equivalence in 2006 and 2010 that has led to two beef plants begin exporting to the U.S. *(10)*. Since 2008, U.S. imports of meat products from Honduras have increased at an estimated 44%. To date, Honduras is eligible to export meat and meat products to the U.S. with an estimated economic impact of approximately $10 million per year *(10)*. Additionally sanitary and phytosanitary capacity building has helped Honduran organizations meet USDA Animal and Plant Health Inspection Service (APHIS) mitigating measures required for exporting peppers and tomatoes among other produce, to the U.S. and has resulted in imports by the U.S. of over $14.1 million in fresh peppers since 2007 *(10)*.

In Honduras, the National Service of Plant and Animal Health (SENASA) through the division of food safety, issues all the regulatory rules and procedures governing the inspection, certification and approval for the operation of the slaughterhouses, packing, storage, and processing of: meat products, aquaculture and fisheries, dairy, beekeeping, fruit, vegetables and animal feed factories, of national origin or imports in reference to the Codex Alimentarius regulations *(4)*. The Codex Alimentarius guidelines are international guidelines for quality and food safety, such as Good Agricultutal Practices (GAP), Good Manufacturing Practices (GMP), and Sanitation Standard Operating Procedures (SSOP) *(25)*.

Honduras Meat's Inspection System

Honduras' meat inspection system is administered by the Official Inspection Service of Animal Origin Products (SIOPOA), an agency within the National Service of

Plant and Animal Health (SENASA), Agriculture and Livestock Secretariat (SAG) *(3)*. SIOPOA has direct authority over all meat establishments including those certified to export meat to the United States. SIOPOA also has direct authority over the government of Honduras' National Laboratory of Residue Analysis (LANAR). This government laboratory performs both chemical and microbiological analytical testing of meat products *(3)*. SIOPOA employs veterinarians and auxiliary inspectors to carry out the responsibility of its domestic and export meat inspection programs including related enforcement activities. All inspection personnel assigned to establishments certified to export meat to the United States are full-time government employees receiving no compensation from either industry or establishment personnel *(3)*. Inspection personnel can hold outside employment provided it does serve as a conflict of interest with their official inspection duties. SIOPOA also has authority over live animal matters in Honduras relative to livestock and poultry movement controls and diseases*(3)*. Meat export certificates are controlled by the Chief Veterinary Officer (CVO) and are signed and distributed on an as-needed basis to the official inspection personnel stationed at the certified establishments *(3)*. The official inspection personnel In Honduras are authorized to enforce the government meat inspection legislation and U.S. import requirements including animal health and welfare, control of animal disease, veterinary medicines, and the production of safe foods of animal origin. SIOPOA, with the assistance of the regulatory enforcement group of SENASA has the authority to suspend and delist certified establishments to prevent the export of unsafe meat to the United States *(3)*.

As mentioned before Honduras has two beef processing plants that export beef products to the U.S *(3)*. The Veterinarians-in-Charge (VICs) in the two establishments certified as eligible to export to the United States maintain physical control of all assigned government seals and stamps. At each certified meat establishment, the VIC has the authority to cease the establishment's production operations any time the wholesomeness and safety of the product is jeopardized. The government of Honduras has the organizational structure and staffing to ensure uniform implementation of U.S. import requirements *(3)*. Honduras' inspection system is audited against FSIS requirements in these two plants *(3)*. The FSIS requirements include daily inspection in all certified establishments, humane handling and slaughter of animals, the handling and disposal of inedible and condemned materials, species verification testing, and FSIS' requirements for HACCP, SSOP, testing for generic *E. coli* and *Salmonella* species *(3)*.

Honduras and U.S. Trade

The U.S. is the chief trading partner for Honduras, supplying 46.2 percent of Honduran imports and purchasing 33.4 percent of Honduran exports in 2011 (excluding maquila trade) *(8)*. Bilateral trade between the two nations totaled $10.6 billion in 2011. U.S. exports to Honduras continued to perform well in 2011 reaching $6.1 billion, an increase of 33% over 2010 *(8)*.

On August 5, 2004, the United States signed the Dominican Republic-Central America-United States Free Trade Agreement (CAFTA-DR) with five Central American countries (Costa Rica, El Salvador, Guatemala, Honduras, and Nicaragua) and the Dominican Republic *(11)*. CAFTA-DR aims to eliminate most tariffs and barriers for

U.S. goods destined for the Central American market, also provide protection for U.S. investments and intellectual property, and create more transparent rules and procedures for conducting business *(11)*. CAFTA-DR also aims to eliminate intra-Central American tariffs and facilitate increased regional trade, benefiting U.S. companies manufacturing in Honduras. With CAFTA-DR implemented, about 80% of U.S. goods now enter the region duty-free, with tariffs on the remaining 20 percent to be phased out by 2016 *(11)*.

According to the U.S. Department of State, since 2009 Honduras export partners include: U.S. (40.9%); El Salvador (8.6%); Guatemala (7.2%); Germany (7.0%); Nicaragua (4.6%); and Belgium (4.3%). The major import partners include: U.S. (33.9%); Guatemala (10.5%); Mexico (6.8%); El Salvador (6.2%); China (4.7%); and Costa Rica (4.6%) *(8)*. The Office of the U.S. Trade Representative also reported U.S. exports of agricultural products to Honduras total $461 million in 2010 *(8)*. Leading products include: coarse grains ($67 million); soybean meal ($57 million); rice ($46 million); and red meats fresh/chilled/frozen ($45 million) *(8)*. United States imports of agricultural products from Honduras totaled $384 million in 2010. Leading products include: bananas and plantains ($176 million); coffee (unroasted) ($77 million); and fresh fruit ($44 million) *(8)*.

Some of Honduras' exports to the U.S. include fresh produce such as cantaloupes, cucumbers, and bell peppers. In 2011, Honduras exported to the U.S. 100,695 MT of cantaloupes; 22,345 MT of cucumbers; 3,386 TM of bell peppers *(12)*.

Mexico Agricultural Industry

Mexico is a large populous Spanish-speaking country located in North America *(7)*. Unlike Honduras, Mexico's economy doesn't rely as heavily on agriculture *(7)*. In Mexico, agriculture makes up an estimated 5% of the country's gross domestic product (GDP), with its major agricultural products consisting of corn; wheat; soybeans; rice; beans; cotton; coffee; fruit; tomatoes; beef; poultry; dairy products; and wood products *(7)*.

In Mexico, the National Health Services of Mexico (SENASICA) as part of the Agriculture, Livestock, Rural Development, Fisheries and Food Secretariat (SAGARPA), has guidelines on what is allowed, regulated and prohibited to bring across the Mexican border *(13)*. SENASICA's mission is to regulate, manage and promote the activities of health, safety and food quality, reducing the risks inherent in agriculture, livestock, aquaculture, and fisheries for the benefit of producers, consumers and industry. SENASICA regulates and oversees all the Phytosanitary regulations to import and export produce in Mexico *(13)*.

Mexico and U.S. Trade

According to the Office of the U.S. Trade Representative, Mexico is currently the 3rd largest trading partner in goods with the U.S. A total of $393 billion in total goods was traded during 2010 *(9)*. Exports totaled $163 billion; imports totaled $230 billion *(9)*. On January 1, 1994, the North American Free Trade Agreement between the United States, Canada, and Mexico (NAFTA) was signed *(9)*. NAFTA created the world's largest free trade area, which now links 450 million people producing $17 trillion worth of goods and services *(9)*. The agreement requires that sanitary and phytosanitary standards for trade be scientifically based, and increases cooperation regarding the environment and labor *(25)*.

United States exports of agricultural products to Mexico totaled $11.8 billion in 2010, the 3rd largest U.S. agricultural export market in the world *(9)*. Leading products include: coarse grains ($3.1 billion); red meats; [fresh/chilled/frozen] ($2.2 billion); soybeans ($1.1 billion); and wheat ($795 million) *(9)*. United States imports of agricultural products from Mexico totaled $13.6 billion in 2010, the 2nd largest U.S. supplier. Leading categories include: fresh vegetables ($3.6 billion); fresh fruit (excluding bananas) ($2.3 billion); wine and beer ($1.6 billion); and snack foods (including chocolate) ($1.3 billion) *(9)*.

Some of Mexico's exports to the U.S. include fresh produce like cantaloupes, bell peppers, cucumbers, tomatoes, and leafy greens. In 2011, Mexico exported to the U.S. 122,968 MT of cantaloupes; 468,859 MT of cucumbers; 120,591 MT of leafy greens; 307,809 MT of bell peppers; 1,238,064 MT of tomatoes *(12)*.

Salmonella

Background

The bacterium *Salmonella* was first discovered by an American bacteriologist, D. E. Salmon in 1886 *(17)*. *Salmonella* species are a group of rod-shaped, Gram-negative, non-spore forming, facultative anaerobe bacteria *(17, 41, 70)*. *Salmonella's* entire genera have currently more than 2,500 serotypes *(1, 70)*. *Salmonella* is currently divided into two species, *Salmonella enterica* and *Salmonella bongori*, which share a high degree of genetic similarity *(70)*. The majority of the serotypes that are pathogenic to humans are part of the species *S. enterica (34, 70)*. *Salmonella* are facultative intracellular bacteria that can be found within a variety of phagocytic and non-phagocytic cells *in vivo (41)*. *Salmonella* species cause illness by means of infection, meaning the microorganism grows and multiplies in the host's body and establish itself in or on the cells or tissue of the host *(17)*. *Salmonella* multiply precisely in the small intestine, colonizing and subsequently invading the intestinal tissues, producing an enterotoxin that causes an inflammatory reaction and diarrhea *(17)*. *Salmonella can* overcome the body's natural defense system by internalizing in macrophages. By this, *Salmonella* enters the blood stream and/or the lymphatic system, causing severe illnesses *(17, 41)*. Overall, the ability of *Salmonella* to survive in a variety of host cells is vital to its success as a human pathogen *(41)*. *Salmonella* species cause a variety of disease syndromes such as gastroenteritis, septicemia, bacteremia and typhoid fever. The most common disease syndrome is a mild to moderate gastroenteritis with symptoms like: diarrhea, abdominal cramps, vomiting, and fever *(1, 17, 28, 34)*. The symptoms are usually self-limiting and resolve within 2 to 7 days. Children, elderly and immunocompromised patients are the most susceptible to severe complications by salmonellosis *(1, 17, 34, 71)*. Foodborne Nontyphoidal *Salmonella* can cause gastroenteritis, bacteremia, and subsequent focal infections *(1)*. In developing countries however, Nontyphoidal salmonellosis is more commonly a severe gastroenteritis with up to 40% of cases accompanied by septicemia in some outbreaks and up to 30% mortality rate *(17)*. *Salmonella* is a ubiquitous microorganism that harbors in the gastrointestinal tract of animals as the primary reservoir *(17, 39, 41, 70)*. *Salmonella* is commonly found in the environment and has various transmission pathways, by which it can be spread to human, animals, foods and the environment *(17, 70)*. A few transmission pathways would include: direct contact with animals (e.g., reptiles and birds); direct personal contact; nosocomial transmission; waterborne transmission; and foodborne transmission *(1)*. Continuous growth of industrialization in animal science and the increasing changes in food habits contribute to expand *Salmonella* worldwide *(1, 39, 58)*. In most human cases, salmonellosis occurs via cross-contamination of meat in slaughterhouses, butcher shops, and, in particular, in people's kitchen during food preparation *(58, 64)*. *Salmonella* also have multiple-antimicrobial agent-resistant strains that are often involved in major outbreaks *(1, 17, 64)*. For instance, strains of *Salmonella* serotype Typhimurium DT104, are increasingly causing food-associated outbreaks of severe gastrointestinal infection and are commonly resistant to at least five and sometimes more antibiotics *(1, 17)*. Food products, consisting of meat and raw milk products are commonly associated in outbreaks attributed to *Salmonella* serotype Typhimurium DT104. Also, direct contact with livestock has also been reported to be a risk factor for human infection *(17, 64)*. Evidently, the increasing role of antibiotic-resistant *Salmonella* is of considerable concern, that is continuously discussed and studied by scientists and governments *(17)*. Traditionally, it was accepted that most cases of human salmonellosis came from foodstuffs of animal origin, but there has also been an increase of *Salmonella*-contamination of produce *(1, 18, 38, 49)*.

Salmonella Surveillance Worldwide

Although there are more than 2,500 serotypes of *Salmonella enterica* identified, most human infections are limited to a small number of *Salmonella enterica* serotypes *(39)*. A study conducted by Hendriksen et al., identified the 15 most frequent serotypes of *Salmonella* in 37 countries, between the years of 2001 to 2007 *(39)*. The regions were the 37 countries were located are: Africa, North America, Asia, Oceania, Europe, and Latin America. The study showed that *Salmonella* serotype Enteritidis and *Salmonella* serotype Typhimurium are the most common as well as second most common serotypes, respectively, in all regions in the study except in North America and Oceania. In North America and Oceania, the most common serotype was *Salmonella* serotype Typhimurium, followed in second by *Salmonella* serotype Enteritidis *(39)*. Additionally the authors revealed that in Latin American countries, *Salmonella* serotype Newport was common and *Salmonella* serotype Agona was very frequent as well *(39)*. One interesting fact highlighted, was how new strains can be introduced to different regions through international travel, human migration, animal feed, food, and livestock trade. Therefore, this could potentially lead to a shift in the prevalence of specific serotypes in human and animal populations worldwide *(39)*. One could state that the failure to control *Salmonella* in one country could potentially affect another country; hence the importance of a global *Salmonella* surveillance system *(39)*. Authors concluded, there is great complexity in the global epidemiology of *Salmonella*. There is an urgency to improve the monitoring of serotypes with high epidemiological importance and implement control measures throughout the food production chain to control their spread *(39)*.

Salmonella is also an important cause of travel-associated diarrhea *(42)*. It is widely accepted that a high index of *Salmonella* infections in the U.S. are due to international travel *(42)*. In a study conducted by Johnson et al., cases of *Salmonella* infection were reviewed, 23,712 case-patients with known travel status were questioned, 2,659 (11%) reported to have traveled outside the U.S. in the 7 days before the illness due to *Salmonella* began *(42)*. The cases described, by Johnson et al., were reported to the Centers for Disease Control and Prevention's (CDC) Foodborne Diseases Active Surveillance Network (FoodNet) from 2004 to 2008 *(42)*. Among the case-patients who had traveled, 1,645 visited a single destination and 107 countries were reported as visited. The most common reported destinations were: Mexico (38%); India (9%); Jamaica (7%); the Dominican Republic (4%); China (3%); and the Bahamas (2%). Also a comparison between travel to higher-income and lower-income countries was made. A higher proportion of travelers with an invasive disease (33%) had traveled to low-income countries, when compared to travelers who had traveled to high-income countries (3%) (*3)*. The four most common serotypes, regardless of travel status reported, include: *S.* Enteritidis (19%); *S.* Typhimurium (14%); *S.* Newport (9%); and *S.* Javiana (5%) (*3)*. *Salmonella* serotype Agona was frequently reported in Latin America. These results agreed with those of Hendriksen et al. *(39)*. Johnson et al. concluded that travel-associated *Salmonella* infections were more commonly reported from travelers to lower-income countries, and that infections in these travelers were more likely to result in hospitalization and invasive disease *(42)*.

In a study conducted by Pires et al. in 2011, different food sources were attributed to human foodborne illness in Latin America and the Caribbean using data from different outbreak investigations *(62)*. The data covered the period from 1993 to 2010, and the study was only focused on bacterial pathogens *(62)*. The study showed the most important sources of disease reported varied in the two decades (1990s and 2000s). In the 1990's, meat and dairy products were the most important food sources with *Salmonella* among the most frequently reported pathogens. Whereas, eggs and vegetables were the most important food sources in the 2000's with *Salmonella* also among the most frequently reported pathogens. The country of Cuba reported *Salmonella* was responsible for the majority of their outbreaks. *Salmonella* serotype Enteritidis was the most common serotype, having been associated with 8.5% of the *Salmonella* outbreaks in the study period *(62)*. *Salmonella* and *Vibrio cholera* were the most frequent pathogens associated with deaths, in Peru, Chile, Mexico and Nicaragua *(62)*. Vegetables and meat were among the top food sources associated with fatal foodborne illness *(62)*. In this study the location of the source responsible for the outbreak was identified, whether there were general outbreaks or household outbreaks. In the case of *Salmonella* outbreaks, they estimated that 38.4% of outbreaks that occurred at home could be attributed to eggs *(62)*. In contrast meat and vegetables appeared to be more important in general outbreaks than in households. This study highlighted the importance of the implementation of an effective food safety surveillance programs in Latin America and the Caribbean, that will allow for the identification of food safety problems and investigation of sources of disease *(62)*.

Salmonella Prevalence in the United States

Salmonella is a leading cause of foodborne illness in the U.S. *(18, 28)*. In 2007, the U.S. Department of Agriculture-Economic Research Service (USDA-ERS) estimated there were 1.4 million cases of salmonellosis that cost the U.S. 2.5 billion dollars in loss of productivity and medical costs. Because there has been a higher demand for fresh produce in the U.S., greater volumes of produce are being imported from Latin American countries, such as Mexico and Honduras. It is expected that the number of foodborne illness like salmonellosis will increase in the coming years. The globalization of the food supply has resulted in unique food safety challenges *(38)*.

In 2012 the Centers for Disease Control and Prevention (CDC) reported in the Morbidity & Mortality Weekly Report, *Salmonella* is the leading cause of bacterial foodborne outbreaks in the U.S. *(36)*. Outbreaks linked to a single pathogen totaled 479, with Norovirus responsible for 49% of outbreaks and 46% of illnesses. *Salmonella* was the second most common pathogen and the leading cause of bacterial foodborne illness, blamed for 23% of outbreaks and 31% of illnesses *(36)*. According to the report which compiled cases from 2008, the most recent year for which data were complete, the top commodities causing outbreaks were poultry (15%); beef (14%); and finfish (14%), while the top commodities to which outbreak-related illnesses were attributed were fruits and nuts (24%); vine-stalk vegetables (23%); and beef (13%). Additionally, *Salmonella* was the greatest cause of outbreak-related hospitalizations, causing 62% of total hospitalizations reported, as well as the etiological agent with most deaths associated. The report also highlighted that among the 108 *Salmonella* outbreaks with a serotype reported, *S.* Enteritidis was the most common serotype, causing 29 (27%) confirmed, single-etiology outbreaks. Seventeen multistate outbreaks were reported in 2008, with nine linked to *Salmonella*. Of the total outbreaks, six food products were involved: cantaloupe, cereal, ground turkey, ground white pepper, jalapeño and serrano peppers, and peanut butter and peanut paste *(36)*.

Several common *Salmonella* serotypes are found in US beef and animal products. In 2000, Dargatz et al. determined the prevalence of *Salmonella* in calf operations in the U.S. *(29)*. Overall, 22 *Salmonella* serotypes were recovered from 1.4% (70 of 5049) samples. The 5 most common serotypes were as follows: *Salmonella* serotype Oranienburg (21.8% of isolates), and *Salmonella* serotype Cerro (21.8%), followed by *Salmonella* serotype Anatum (10.3%), *Salmonella* serotype Bredeney (9.0%), and *Salmonella* serotype Mbandaka (5.1%) *(29)*. A total of nine isolates, showed multiple antibiotic resistance, most commonly to streptomycin and sulfamethoxazole *(29)*.

In 2008, also in the U.S., Kunze et al. investigated the burden of *Salmonella enterica* in harvest-ready beef cattle *(50)*. *Salmonella enterica* was recovered from 55.6% of 1,681 samples. The prevalence on hides and in feces was 69.6% and 30.3%, respectively *(50)*. A total of twenty-two serotypes were identified in this study; the most common serotype discovered was *S. enterica* serotype Anatum (25.5%), followed by *S. enterica* serotype Montevideo (22.2%), and *S. enterica* serotype Cerro (12.5%) *(50)*.

Salmonella Contamination of Produce

Traditionally most foodborne outbreaks are associated with foods of animal origin (poultry, eggs, beef); however, recent investigations have identified fruits and vegetables as the source of many recent foodborne outbreaks in the last decade *(14, 18, 27, 35, 38, 49)*. There are many potential sources by which produce can become contaminated; some are at the pre-harvest stage and others at a post-harvest stage. Some outbreaks can also be attributed to food preparation and the food preparing environment *(38)*. An example of a contaminant in both the pre-harvest and the post-harvest stage can be water, which can be contaminated from the run-off of nearby pastures or water used for irrigation *(49)*. Water of unknown microbiological quality can become an important hazard for food crops *(51)*. In addition, the feces of wild animals could be a source of contamination at the pre-harvest stage. Some examples of contamination at the post-harvest stage are storage, rinsing, cutting. Cut surfaces on produce can be a target for pathogenic bacteria like *Salmonella (18, 27, 38, 49, 52)*. Recycled rinsing water, transport vehicles, and processing equipment are other examples of post-harvest contamination *(27, 52)*.

Different *Salmonella enterica* serotypes can colonize seeds, sprouted seeds, leaves and fruits of a variety of plant species *(18, 27, 51)*. According to a study conducted by Berger et al in 2010, the association of *Salmonella* with fresh produce appears to be serovar-specific involving flagella, pili, cellulose, and O antigen capsule *(18)*. According to this study, the mechanisms of adhesion among the different serotypes seem to be different *(18)*. For example, authors reported that in the serotypes Typhimurium and Newport, the pilus curli is of importance in the adhesion to alfalfa sprouts *(18)*. Barak et al. showed that the O antigen capsule and the cellulose synthesis play a role in adhesion of serotype Enteritidis *(16)*. The curli, cellulose, and capsule are all regulated by one gene known as *AgfD*; which, is known to play an important role in *Salmonella's* environmental fitness *(18). Salmonella* biofilm formation occurs because of the curli and cellulose role in the formation of a cellular matrix. *Salmonella* serotypes forming strong biofilms were found to have stronger adhesion to plant leaves *(18)*.

Aside from adhering to plant surfaces, some studies have also shown some *Salmonella* serotypes can invade plant tissue *(18, 49)*. Because *Salmonella* can invade plant tissues such as tomatoes and lettuces, poses a challenge for the produce industry *(18, 38, 49, 51)*. After *Salmonella* is internalized by a plant, sanitizing treatments like chlorine or ozonated water are ineffective against the internalized pathogen. The only treatment currently available to eliminate internalized *Salmonella* from produce is irradiation because of its ability to penetrate tissues. Unfortunately, there exists a lack of public approval of food irradiation because of its use can produce off flavors, colors and odors and destroy some nutrients when used in produce *(38)*. It has also been observed that the *Salmonella* colonization level can be different for various serotypes and among different species of the host plant, which could indicate that the colonization level is determined by various factors *(49)*. According to Krtinic et al. just one CFU is enough to colonize the internal part of a plant five days after inoculation which is a disturbing result concerning food safety *(49)*.

Wide spectrums of produce vehicles are associated with *Salmonella* infections worldwide *(18, 38, 49)*. Tomatoes and melons, for example have been linked to large-scale outbreaks in the U.S. *(2, 18, 19, 27, 35)*. Jalapeño and serrano peppers were also linked to a large multi-state outbreak of *Salmonella* serotype Saintpaul in 2008 *(43, 47, 49, 56)*. Leafy greens including different kinds of lettuce have also been linked to several outbreaks *(24, 48, 57, 75)*. Several other outbreaks of *Salmonella enterica* have occurred in Europe, with several produce vehicles associated such as basil and sprouted seeds *(18, 49, 60)*.

Numerous outbreaks of *Salmonella* associated with melons (cantaloupes, honeydew, and watermelon) have occurred over the years *(2, 35, 38, 60)*. Melon rinds are often contaminated with *Salmonella.* Contamination usually occurs when the melon is cut, exposing the edible portions *(35, 38)*. Because melons are naturally rich in sugars and other nutrients along with a pH close to neutral (7.0), *Salmonella* can grow, especially when melons are not stored properly *(38)*. Contamination of melons can occur from multiple sources; however it has been shown that the majority of *Salmonella* contamination resulted from rinds being inoculated during immersion in contaminated wash water in post-harvest facilities *(38)*. In the case of cantaloupes, they are difficult to decontaminate, because the surface contains lenticles that provide areas for attachment and protection against sanitizers *(38)*. Prevention of melon contamination with *Salmonella* is a critical control point. Many U.S. producers have implemented guidelines in prevention of melon contamination. However, a large portion of melons that are imported from Latin American countries may not follow U.S. guidelines. For instance, this was demonstrated by the 2008 outbreak due to cantaloupe imported from Honduras *(38)*.

Tomatoes have been a common food product involved in many *Salmonella* outbreaks *(18, 19, 38)*. Studies have shown different sources by which tomatoes become contaminated. Some sources would include contaminated irrigation water and contaminated wash water *(19, 38)*. Naturally, tomatoes have a fairly acidic pH (4.0-4.6) depending on the ripeness of the tomato. With this said, not all *Salmonella* serotypes can survive these acidic conditions, but some serotypes can grow at a pH as low as 3.7, which means they can grow on acidic fruit as tomatoes. Some serotypes commonly linked to tomatoes are Montevideo, Newport and Hadar. If a tomato is contaminated, it may be difficult to eliminate *Salmonella* if it has been internalized *(73)*. *Salmonella* internalization by tomatoes and other fruits and vegetables, creates a significant challenge for food safety because *Salmonella* is physically protected from chemicals and sanitizers. Also, *Salmonella* on stem scars and cracks in the skin can survive better than on the smooth skin *(38, 51)*.

***Salmonella* Outbreaks Associated with Produce**

Several outbreaks of *Salmonella* have been associated with contaminated produce in the last couple of decades as *(18, 27, 38, 49)*. Many *Salmonella* outbreaks have been linked to fruits and vegetables imported from several Latin American countries as mentioned before *(2, 44, 61, 62)*.

An outbreak of typhoid fever occurred in the winter of 1998-1999 in Florida with at least 16 people sickened after consuming fruit shakes made with frozen mamey, a tropical fruit *(44)*. The frozen mamey was prepared in plants in Guatemala and Honduras. Although this outbreak was a typhoid fever outbreak, this was the first typhoid fever outbreak in the U.S. caused by commercially imported food. This outbreak introduced a new pattern of foodborne outbreaks, characterized by cases scattered across counties, states, and nations *(44)*.

Three multistate outbreaks occurred in the U.S. in the spring of consecutive years during 2000-2002 *(2)*. The outbreaks were of *Salmonella* serotype Poona infections associated with eating cantaloupe imported from Mexico *(2)*. In each outbreak, the isolates had indistinguishable pulse-field gel electrophoresis (PFGE) patterns. The outbreaks involved residents of 12 U. S. states and Canada. Because of the 2000 and 2001 outbreaks, the FDA conducted an on-farm investigation in Mexico and concluded that there were no measures taking to minimize microbial contamination in the growing, harvesting, packaging, and cooling of the cantaloupe *(2)*.

In 2004, an international outbreak of *Salmonella* serotype Thompson infections was associated to imported rucola lettuce from Italy *(57)*. The outbreak started in Norway, with a cluster of three cases of *Salmonella* serotype Thompson infections and by December 31st, 21 cases had been reported. Because of the outbreak in Norway, a request was sent to Enter net (the International Surveillance Network for Enteric infections *Salmonella* and VTEC O157), involving all 15 countries of the European Union plus Australia, Canada, Japan, South Africa, Switzerland, and Norway, to ascertain whether any other country had noticed an increase in notifications of *Salmonella* serotype Thompson infections *(57)*. Sweden and England reported an increase with a total of 17 and 46 cases, respectively. Italy reported an outbreak of *Salmonella* serotype Thompson in a school, which affected 57 children. Switzerland also reported a peak in the Italianspeaking region of the country. During the outbreak investigation other pathogens were linked to the implicated rucola lettuce, which indicated a massive contamination, possibly caused by irrigation with nonpotable water. This outbreak is a clear example of how the large and increasing global trade in fresh produce, can potentially cause large international outbreaks like this one *(57)*.

In a report from the CDC Morbidity and Mortality Weekly Report, in 2005–2006, four large multistate outbreaks of *Salmonella* infections associated with eating raw tomatoes at restaurants occurred in the U.S. *(19)*. The four outbreaks resulted in 459 culture-confirmed cases of salmonellosis in 21 states and Canada *(19)*. The tomatoes implicated on the outbreaks had been supplied to restaurants either whole or precut from tomato fields in the states of Florida, Ohio, and Virginia. The serotypes implicated in these outbreaks were *Salmonella* serotype Newport, Braenderup and Typhimurium *(19)*. These recurrent multistate outbreaks were a clear example that the tomato-growing environment is an ongoing source of contamination of tomatoes *(19)*. There were many environmental sources of *Salmonella* contamination of tomatoes including feces from domestic or wild animals (e.g., reptiles, amphibians, or birds) and contaminated habitats, such as ponds or drainage ditches *(19)*.

In 2007, an international outbreak of *Salmonella* serotype Seftenberg was associated to imported fresh basil from Israel *(60)*. Thirty-two cases were identified in England and Wales. Nineteen cases were also reported from Scotland, Denmark, the Netherlands, and the U.S. *(60)*. One important characteristic of this outbreak was that the strain was not commonly found in the countries affected. The identification of the international outbreak was possible due to sharing information, including PFGE profiles, among all the countries affected via the PulseNet/Enter-Net networks *(60)*.

In 2008, a multistate outbreak of *Salmonella* serotype Saintpaul was initially associated with tomatoes, but further epidemiological and microbiological investigations linked the outbreak to jalapeño and serrano peppers imported from Mexico *(43, 47, 56)*. The multistate outbreak spread to 43 states, the District of Columbia, and Canada with a total of 1,442 cases reported *(43, 47)*. The majority of the affected persons became ill in May and June, with the highest incidence reported in the states of New Mexico and Texas *(43, 47)*. Early in the outbreak investigation there was an epidemiological association between illness and the consumption of tomatoes, but cases continued to appear after an import alert was placed on tomatoes coming from Mexico. Further traceback investigations showed that jalapeño and serrano peppers were associated with illness *(43, 47, 56)*. Table 2.1 condenses all the recent *Salmonella* outbreaks associated to produce.

Table 2.1Recent Outbreaks of Salmonella Associated to Produce Contamination.

YEARS	**PRODUCE TYPE**	***SALMONELLA* SEROTYPE**	**LOCATION**	**PLACE OF ORIGIN**
1998-1999	Mamey	*Salmonella typhi*	Florida, US	Guatemala and Honduras
2000-2002	Cantaloupe	*Salmonella* Poona	Multistate outbreak in the US and Canada	Mexico
2004	Rucola lettuce	*Salmonella* Thompson	Norway, Sweden, England, Switzerland, Italy	Italy
2005-2006	Tomatoes	*Salmonella* Newport, Braenderup and Typhimurium	Four multistate outbreaks	Florida, Ohio and Virginia
2007	Basil	*Salmonella* Seftenberg	England and Wales, Scotland, Denmark, the Netherlands, United States	Israel
2008	Jalapeño and Serrano peppers	*Salmonella* Saintpaul	Multistate outbreak in the US and Canada	Mexico

Salmonella Contamination of Beef

The majority of meat safety issues resulting in immediate consumer health problems and recalls of contaminated products are associated with microbial and especially bacterial pathogens *(37, 68)*. Major causes of concern and product recalls associated with fresh meat products are *E. coli* O157:H7 and related enteric pathogens such as *Salmonella (22, 37, 55, 68)*. *Salmonella* continues to be a pathogen of current concern in meat safety. *Salmonella* poses a threat to the food animal industry as an evolving pathogen *(68)*. Over the years, *Salmonella* has evolved, becoming more virulent, presenting high levels of antibiotic resistance such as *Salmonella* serotype Typhimurium DT104 or *Salmonella* Newport R-type MDR-Amp C strains *(41, 45, 54, 68)*. Despite being the target of control efforts for many decades, *Salmonella* still remains involved in large number of illnesses and outbreaks *(21, 28, 34, 35, 37, 54, 55, 58, 68, 72)*.

Generally, most cases of human salmonellosis are linked to foods of animal origin like meat, poultry, eggs, and milk *(34, 39, 49, 54, 55, 58, 68, 74)*. Meyer et al. stated, between 80% to 90% of the Salmonellosis cases in industrialized countries are linked to the consumption of foods of animal origin *(55)*. Since *Salmonella* can colonize the gastrointestinal tract of animals without causing any clinical symptoms, poses a food safety issue because carcasses contaminated with *Salmonella* at the time of slaughter may not show signs of contamination *(54, 55)*. Beef carcasses testing positive for *Salmonella* can lead a food safety issue if not eliminated throughout the fabrication process *(54)*.

Salmonella Prevalence in Beef

In a study conducted by Bacon et al. in 2002, the prevalence of *Salmonella* was determined on cattle hides and on carcasses after processing from 8 commercial abattoirs in the U.S. *(15)*. The overall prevalence of *Salmonella* on hides, was 15.4%; whereas, the prevalence after dehiding and other slaughtering/dressing processes, including the application of decontamination treatments, was, as expected, reduced to 1.3% on carcass surfaces *(15)*.

A study conducted in 2003 by McEvoy et al. determined the prevalence of *Salmonella* spp. in bovine fecal, rumen and carcass samples at a commercial abattoir in Ireland *(54)*. During the 12-month period of this study, *Salmonella* was isolated from 2.0% of the fecal samples, 2.0% of the rumen samples and from 7.6% of the carcass samples *(54)*. *Salmonella* serotype Dublin was the predominant serotype, being isolated from 72% of positive samples. *Salmonella* serotype Typhimurium and *Salmonella* serotype Agona were each isolated from 14% of positive samples. All *Salmonella* serotype Typhimurium isolates were DT104 *(54)*.

Rivera-Betancourt et al. in 2004 reported the prevalence of *Salmonella* and other foodborne pathogens in two geographically distant commercial beef processing plants in the U.S. *(65)*. The results of the study showed 23.3% of beef carcasses in the plant A and 26.8% of beef carcasses in plant B were *Salmonella* positive *(65)*. The overall results of this study suggested that there were regional differences in the prevalence of pathogens on hides of cattle presented for harvest at commercial beef processing plants. Authors concluded while hide data may reflect the regional prevalence, carcass data is indicative of differences in harvest practices and procedures in these plants *(65)*.

In another study, conducted by Ruby et al. in 2007, *Salmonella* prevalence was determined in three abattoirs in the U.S. *(66)*. *Salmonella* was found in 45% (N= 5,355)of beef carcasses and it was 0.5% positive after antimicrobial treatment, showing a clear reduction *(66)*. Also in 2007, Fluckey et al., determined patterns of cross-contamination and antibiotic susceptibility of microorganisms commonly associated with cattle. *Salmonella* was identified in 33.9% (n = 20) of the fecal samples and on 37.3% (n = 22) of the hides of beef cattle before they were shipped to a commercial abattoir. Once at the abattoir, the proportion of hides from which *Salmonella* was isolated increased ($P < 0.001$) to 84.2% (48 hides). Authors showed 97% (n = 101) of *Salmonella* isolates were resistant to at least one antimicrobial drug; however, only 4.0% were resistant to two or more *(33)*. In addition in 2007, Hernandez San Juan et al., determined the microbiological conditions during the slaughter process of a municipal slaughterhouse in Hidalgo, Mexico *(40)*. For the samples collected, *Salmonella* was detected in 31% of pork line samples and 11% of the beef line samples. Authors concluded that microbial counts present in carcasses, utensils and personnel indicated poor hygienic conditions in the slaughtering establishment and that there is a need to implement and maintain good manufacturing practices (GMP) to achieve meat safety *(40)*.

A study conducted in 2008, by Dewell et al., determined the risk associated with transportation and lairage on hide contamination with *Salmonella enterica* in finished beef cattle at slaughter *(31)*. The study showed cattle transported for long distances had twice the risk of having positive hide samples at slaughter compared with cattle transported shorter distances (RR, 2.3) *(31)* Cattle held in lairage pens contaminated with feces had twice the risk of having positive slaughter hide samples, when compared with cattle held in clean pens (RR, 1.8). According to this study, the variables associated with transportation and lairage can impact the presence of *Salmonella* on the hides of cattle at slaughter *(31)*. Also in 2008, Brichta-Harhay et al., determined *Salmonella* and *Escherichia coli* O157:H7 prevalence on hides and carcasses of cull cattle presented for slaughter at four geographically distant regions in the United States *(26)*. *Salmonella* was found on hides 89.6% (95% confidence interval [CI], 85.1 to 94.0); pre-evisceration 50.2% (95% CI, 40.9 to 59.5); and post-evisceration 0.8% (95% CI, 0.18 to 1.42) *(26)*. Authors observed pathogen prevalence on hides and carcasses was not significantly affected by the season; however, significant differences were observed between plants with respect to the incoming pathogen load and the ability to diminish hide-to-carcass transfer. In spite of these differences, post-evisceration carcass contamination was significantly reduced ($P < 0.001$) after interventions were applied by the processing plants *(26)*.

In another study in 2009, Bosilevac et al. determined the prevalence of *E. coli* O157:H7 and *Salmonella* in small beef processing plants in the U.S. *(22)*. The overall prevalence of *Salmonella* on hides was 91% and there was a 58% overall prevalence on preevisceration carcasses *(22)*. The authors agreed, that the results obtained are comparable to those observed previously for larger beef processors, showing that smaller beef processors face and address the same challenges as do larger beef processors *(22)*.

In another study in 2011, Bravo et al. determined the prevalence of *E. coli* O157:H7 and *Salmonella* in a vertically integrated feedlot and harvest plant in Mexico *(25)*. *Salmonella* was isolated from 836 of 1,639 (51%) samples tested in the study which included 5 sampling times. The overall *Salmonella* prevalence was 55.56% (105/189) in the feedlot; 91.0% (91/200) in the holding pen; 46.8% (117/250) in feces; 92.4% (231/250) in hides; 49.6% (124/250) in pre-evisceration; 24.8% (62/250) in pre-cooler; and 6.0% (15/250) in cooler. The prevalence of *Salmonella* on hides observed in this study was relatively high during all five sampling times, with more than 60% positives. Similarly, the prevalence at the holding pens did not show important variations, remaining high (more than 80%) during all five samplings times *(25)*. Authors reported that a statistical analysis showed differences by sampling dates, indicating a seasonal effect on *Salmonella* prevalence; however it was not clear why this variation occurred.

Contaminated raw or undercooked red meats like beef can become main vehicless of transmission for *Salmonella (37, 55)*. Although beef is not usually consumed raw, there is a risk of a *Salmonella* infection if the meat is improperly cooked, or if there is cross contamination of *Salmonella* with other foods that are consumed raw *(35)*. In a study conducted by Stopforth et al. in 2006, the authors assessed the microbiological contamination of fresh beef cuts from two processing plants in the U.S. Midwest *(69)*. The authors determined total bacterial load as well as that of indicator microorganisms such as coliforms, and they also assessed the safety of the fresh beef cuts by investigating the incidence of *E. coli* O157:H7 and *Salmonella (69)*. The results of the study indicated that *E. coli* O157:H7 was exclusively isolated from cuts derived from the sirloin area, while *Salmonella* was exclusively isolated from cuts derived from the chuck, rib, and loin areas *(69)*. The authors concluded that contamination of beef cuts may be influenced by the region of the carcass they are derived from *(69)*. The study also suggested that pathogens such as *E. coli* O157:H7 and *Salmonella* are invariably present in fresh beef surfaces and particularly cuts that have been already exposed to primary interventions intended to reduce, control, and/or eliminate such pathogens *(69)*. The beef industry does not have microbiological guidelines for fresh beef cuts, so studies like the one conducted by Stopforth et al. could be used in the establishment of a microbiological baseline for fresh beef cuts *(69)*.

In 2008, Little et al. determined the prevalence of *Salmonella* and *Campylobacter* in raw red meats in the UK during 2003-2005 *(53)*. *Salmonella* was found in 1.3% of all beef samples collected, and *Salmonella* serotype Typhimurium was the most frequent *Salmonella* serotype isolated from meats *(53)*. *Salmonella* serotype Typhimurium DT104/104b isolates exhibited higher rates of multiple drug resistance than other serotypes *(53)*. In another study conducted by Gallegos-Robles in 2009, *Salmonella* presence was tested on fresh beef cuts *(35)*. *Salmonella* was found in 8.0% of all the fresh beef samples collected *(35)*.

In a study conducted by Bosilevac et al. in 2009, the prevalence and characterization of *Salmonella* was determined in commercially produced ground beef in the US *(23)*. In the study the overall prevalence of *Salmonella* strains was 4.2% *(23)*. All *Salmonella* isolates obtained in this study were serotyped and their antibiotic susceptibilities determined and analyzed by pulsed-field gel electrophoresis (PFGE). The most common serotypes identified in this study were *Salmonella enterica* serotypes Montevideo, Anatum, Muenster, and Mbandaka *(23)*. The prevalence of multidrug-resistant (MDR) *Salmonella* was 0.6%. The most common MDR serotypes were *Salmonella* enterica serotypes Dublin, Reading, and Typhimurium. MDR strains had resistance to between 2 and 10 antibiotics *(23)*.

In a study conducted by Pond et al. in 2010, the prevalence of *Salmonella* and *E. coli* O157:H7 was determined as well as a quantification of generic *E. coli* and coliforms in fresh beef and pork products from retail outlets in major cities in Mexico *(63)*. Samples in this study were collected from the most populated cities in Mexico: Mexico City, Monterrey, and Guadalajara. Types of samples included whole beef, ground beef, whole pork, and ground pork *(63)*. In Mexico City, *Salmonella* was not found in beef (whole and ground) but was 2.5% positive (1/40) in whole pork, and 4.2% positive (1/24) in ground pork. In Monterrey, no *Salmonella* was detected in whole pork, ground beef, or ground pork but was 1.14% positive (1/88) in whole beef. In Guadalajara, *Salmonella* was 33.3% positive (2/13) in whole beef and 50.0% positive (7/14) in ground pork *(63)*.

Salmonella **Outbreaks Associated with Beef**

Throughout the last couple of decades several *Salmonella* outbreaks have been associated with contaminated beef *(30, 37, 68)*. Over the years there has been an increase of *Salmonella* serotype Typhimurium DT104 infections. All the outbreaks associated with these serotype were associated with dairy products and contact with animals *(30)*.

An outbreak of multidrug-resistant *Salmonella enterica* serotype Typhimurium Definitive Type 104 linked to commercial ground beef in the Northeastern U.S. in 2003–2004 was investigated by Dechet et al. *(30)*. The outbreak reported by Dechet et al. was the first outbreak in the U.S. that has been associated with ground beef purchased from grocery stores *(30)*. A total of 58 case patients were identified in 9 states by pulse-field gel electrophoresis (PFGE) *(30)*. Isolates were resistant to ampicillin, chloramphenicol, streptomycin, sulfamethoxazole, and tetracycline (R-type ACSSuT). Illness was associated with consuming store-bought ground beef prepared as hamburgers at home and with eating raw ground beef *(30)*. Product traceback linked cases to a single large ground beef manufacturer previously implicated in a multistate outbreak of highly drug-resistant *Salmonella* enterica Newport infections in 2002 *(30)*.

Also in 2007, an outbreak of multidrug-resistant *Salmonella* serotype Newport associated to ground beef affected 42 case patients in California, Arizona, Idaho, and Nevada *(67)*. The case patients consumed multiple types of ground beef products purchased at numerous chain store retail locations. These stores had received beef products for grinding from multiple beef slaughter-processing establishments. Despite detailed ground beef purchase histories both the USDA-FSIS and the California Department of Public Health were unable to identify the source of contamination *(67)*.

In a study published in 2009 by Greig et al. an analysis of foodborne outbreak data reported internationally was made for source attribution *(37)*. The study was conducted using data reported between 1996 and 2005 in four regions based on publicly available reports. Among the 4,093 foodborne outbreaks with accessible and useful information, almost 70% were attributable to *Salmonella*, Norovirus and *E. coli* (46.9, 13.5 and 9.5%, respectively). *Salmonella* serotype Enteritidis was the most frequent *Salmonella* serotype (991 foodborne outbreaks or 24.1% of the total), followed by Typhimurium (270 outbreaks or 6.6%) *(37)*. The most frequently reported food categories were 'Multi-ingredient foods', 'Eggs', and 'Produce' and 'Beef' at the third rank (17.0, 14.3, 12.2 and 12.2% of all outbreaks, respectively) *(37)*. The total number of outbreaks liked to *Salmonella* serotype Enteritidis in beef were 46, *Salmonella* serotype Typhimurium outbreaks in beef were a total of 13, and other *Salmonella enterica* outbreaks in beef were a total 48 in this study *(37)*. The authors concluded that the study highlighted a common lack of high specificity in the association between etiology and food vehicle for both zoonotic and non-zoonotic diseases, suggesting that overall cross-contamination, environmental contamination and food handler contamination maybe common along the food chain *(37)*.

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CHAPTER III

III. BASELINE OF *SALMONELLA* PREVALENCE IN RETAIL BEEF AND PRODUCE FROM HONDURAS AND MEXICO

INTRODUCTION

Human salmonellosis is one of the most frequently occurring foodborne diseases worldwide. According to Bollaerts et al., global estimations of human salmonellosis varies between 14 to 120 per 100,000 people *(21)*. Annually, both developed and developing countries report high numbers of salmonellosis worldwide. Because these reports represent 1 to 10% of the real incidence of this disease, it is hard to estimate the real burden of salmonellosis worldwide *(58)*. It has been estimated that *Salmonella* causes approximately 1.4 million infections, 15,000 hospitalizations, and 400 deaths annually in the U.S. alone *(30, 72)*. The majority of the mentioned cases are considered to be foodborne and the food industry is making considerable efforts to reduce the levels of this pathogen on its products *(72)*.

Salmonella has been associated with recent outbreaks in the U.S. as the result of undercooked or improperly handled beef products *(69)*. In addition, outbreaks associated with multidrug resistant strains like *Salmonella* serotype Typhimurium in beef products have increased *(30, 45)*. It is important to take into consideration that *Salmonella* frequently enters the human food chain during bovine slaughter and carcass processing *(45)*. Therefore, there is a need to control *Salmonella* throughout the whole food chain.

Generally, most cases of foodborne salmonellosis are associated to animal products like beef and poultry *(38, 49)*. However, over the years there has been an increase in the number of *Salmonella* outbreaks linked to fresh produce like cantaloupes, peppers, and tomatoes *(49)*. *Salmonella* also has the ability to be internalized by many produce types, makes it merely impossible for the produce industry to control; therefore, posing a challenge for food safety worldwide *(38, 48, 49)*.

Globalization has caused the international food industry to increase the production and trade of produce and animal products (beef). This globalization has created unique food safety challenges worldwide. The cooperation among international partners, worldwide regulations, and enhanced surveillance systems are of necessity to help keep food safe.

Determining the prevalence of *Salmonella* in Latin American countries, such as Honduras and Mexico, will allow the establishment of a baseline, which will be useful indicating where the primary areas of concern exist and will ultimately allow for control of foodborne *Salmonella.*

Materials and Methods

Honduras Sampling

Through microbial analyses, the prevalence of *Salmonella* was determined in Honduran retail beef and produce at two types of retail markets (supermarkets and wet markets; which are open city markets) in four major cities of Honduras: San Pedro Sula, Siguatepeque, Tegucigalpa and Choluteca. Each city represents the north, west, central and south part of the country respectively. Samples purchased from each market included whole muscle beef and six types of fresh produce: cantaloupes, cilantro, cucumbers, leafy greens (lettuce), peppers (bell), and tomatoes if available. Samples were also collected from beef carcasses in two separate slaughter facilities in the cities of Catacamas and Siguatepeque. All samples were collected during a 14 month period from February 2011 through April 2012.

Market Beef Sampling

The goal of this study was to determine the prevalence *Salmonella* in the entire country. Four major cities were visited; San Pedro Sula, Siguatepeque, and Choluteca were visited at least one time and the capital of Honduras, Tegucigalpa was visited up to three times. These four cities account for approximately ½ of the country's population.

San Pedro Sula was sampled in the winter season of 2011 in February. Retail beef was collected from both wet markets and supermarkets. For supermarkets, samples included 33 whole beef. For wet markets, samples included 30 whole beef.

Siguatepeque was sampled in the fall season of 2011 in November. Retail beef was collected from both wet markets and supermarkets. For supermarkets, samples included 25 whole beef. For wet markets, samples included 22 whole beef.

Choluteca was sampled in the fall season of 2011 in November. Retail beef was collected from wet markets and supermarkets. For supermarkets, samples included 20 whole beef. For wet markets, samples included 28 whole beef.

Tegucigalpa was sampled in the summer and fall season of 2011 as well as the spring of 2012. Summer samples were taken in June 2011, fall samples were taken in November 2011, and spring samples were taken in April 2012. Retail beef was collected from wet markets and supermarkets. For supermarkets, samples included 160 whole beef. For wet markets, samples included 75 whole beef (Table 3.1).

Table 3.1 Total of retail beef samples collected during sampling months in each city in Honduras.

CITY	TIMES VISITED	MONTH	TOTAL SAMPLES
San Pedro Sula	1	February, 2011	63
Siguatepeque	1	November, 2011	47
Choluteca	1	November, 2011	48
Tegucigalpa	3	June and November, 2011 April, 2012	235
TOTAL	6		N = 393

Carcass Sampling

In addition to the retail beef samples collected in Honduras, beef carcass samples were collected from two beef slaughter plants in the spring of 2012. A total of 90 samples were collected from Plant A in the city of Catacamas, located on the east part of the country. Carcass swabs were taken at 3 locations along the process; 15 from the hides, 15 at pre-evisceration and 15 at post-evisceration. Samples were collected in the morning shift and afternoon shift, making a total of 45 samples per shift and 90 samples in total for plant A. Also a total of 51 samples were collected from Plant B in the city of Siguatepeque, located on the west part of the country. Carcass swabs were taken at 3 locations along the process; 17 from the hides, 17 at pre-evisceration and 17 at post-evisceration. Samples were collected in the morning shift, making a total of 51 samples for plant B (Table 3.2).

Table 3.2 Total of carcass swabs samples collected in two beef plants located in the cities of Catacamas and Siguatepeque, Honduras. The sampling period was April 2012.

PLANT A/B	SAMPLE NUMBER	CARCASS LOCATION
A	30	Hide
A	30	Pre-evisceration
A	30	Post-evisceration
Total	**90**	
B	17	Hide
B	17	Pre-evisceration
B	17	Post-evisceration
Total	**51**	

All beef carcass samples were surface swabbed using a sponge pre-hydrated with 10 mL of buffered peptone water. All samples were immediately transported back to Texas Tech under cold conditions via commercial air for detection of *Salmonella* and Non-O157 Shiga toxin producing *Escherichia coli*.

Market Produce Sampling

In Tegucigalpa we collected a total of 291 samples, 136 from wet markets and 155 from supermarkets. In the wet markets, samples included 25 cilantro, 26 cucumbers, 21 leafy greens, 33 peppers, and 31 tomatoes. No cantaloupes were available for purchasing. In the supermarkets, samples included 21 cantaloupes, 16 cilantro, 20 cucumbers, 34 leafy greens, 40 peppers, and 24 tomatoes.

In Choluteca we collected a total of 46 samples from wet markets. The samples included 8 cilantro, 10 cucumbers, 8 leafy greens, 10 peppers, and 10 tomatoes. No cantaloupes were available for purchasing.

In Siguatepeque we collected a total of 46 samples from wet markets. The samples included 10 cilantro, 10 cucumbers, 11 leafy greens, 5 peppers, and 10 tomatoes. No cantaloupes were available for purchasing (Table 3.3)

Table 3.3 Total of retail produce samples collected during sampling months in each city in Honduras.

CITY	**TIMES VISITED**	**MONTH**	**TOTAL SAMPLES**
Tegucigalpa	3	June and November, 2011 April, 2012	291
Choluteca	1	November, 2011	46
Siguatepeque	1	November, 2011	46
TOTAL	5		N = 383

Approximately, 50 to 100 whole muscle beef and produce were collected in each city for sampling in each visit for a total of 393 retail beef and 383 produce samples in Honduras. Beef samples were transported in cold conditions to the hotel where they were processed under aseptic conditions, as well as the produce samples. Beef samples were surface swabbed using a sponge pre-hydrated with 10 mL of buffered peptone water and the produce samples were rinsed in buffered peptone water and the sample was collected with a dry-sponge from the rinsate. All samples were immediately transported back to Texas Tech under cold conditions via commercial air for detection of *Salmonella.* Texas Tech has APHIS permits to allow us to bring meat and produce samples into the US for research.

Mexico Sampling

The prevalence of *Salmonella* in Mexican produce was determined at two types of retail markets if available (supermarkets and wet markets; which are open city markets) in four major cities of Mexico: Mexico City, Veracruz, Merida and Cancun. All four cities are major cities in the south central part of the country. Samples purchased in each city included cantaloupes, cilantros, cucumbers, leafy greens (lettuce), peppers and tomatoes if available. All the samples were collected in a 12-month period from April 2011 through March 2012. A baseline of the prevalence of *Salmonella* in beef had already been established by Texas Tech University and was not repeated in this study.

Market Produce Sampling

Cancun was sampled in the spring, summer and winter season of 2011. Spring season includes samples taken in April 2011, summer includes samples taken in August 2011, and winter includes samples taken in December 2011. Retail produce was collected from wet markets. Samples included 17 cantaloupes, 20 cilantro, 19 cucumbers, 21 leafy greens, 26 peppers, and 23 tomatoes. All samples were collected in three separate visits to the city.

Merida was sampled in the spring, summer and winter season of 2011 and the spring of 2012. Spring season includes samples taken in April 2011, summer includes samples taken in August 2011, and winter includes samples taken in December 2011. Retail produce was collected from wet markets. Samples included 32 cantaloupes, 41 cilantro, 41 cucumbers, 39 leafy greens, 30 peppers, and 36 tomatoes. All samples were collected in four separate visits to the city.

Mexico City was sampled in the spring season of 2011. Spring season includes samples taken in April 2011. Retail produce was collected from wet markets. Samples included 5 cantaloupes, 5 cilantro, 5 leafy greens, 5 peppers, and 5 tomatoes. All samples were collected in one visit to the city.

Veracruz was sampled in the spring, summer and fall season of 2011. Spring season includes samples taken in April 2011, summer includes samples taken in June 2011, and fall includes samples taken in September 2011. Retail produce was collected from wet markets. Samples included 24 cantaloupes, 23 cilantro, 20 cucumbers, 26 leafy greens, 25 peppers, and 36 tomatoes. All samples were collected in three separate visits to the city. (Table 3.4)

Table 3.4 Total of retail produce samples collected during sampling months in each city in Mexico.

CITY	TIMES VISITED	MONTH	TOTAL SAMPLES
Cancun	3	April, August, December, 2011	126
Merida	4	April, August, December, 2011 March, 2012	219
Mexico City	1	April, 2011	25
Veracruz	3	April, June, September, 2011	144
TOTAL	11		514

Approximately, 5 to 10 types of produce were collected in each city for sampling in each visit for a total of 514 produce samples in Mexico. Produce samples were transported to the hotel where they were processed under aseptic conditions. Produce samples were rinsed in buffered peptone water and the sample was collected with a dry-sponge from the rinsate. All samples were immediately transported back to Texas Tech under cold conditions via commercial air for detection of *Salmonella.*

Microbial Analysis of *Salmonella*

Salmonella Detection

The *BAX® System PCR Assay* was used for the detection of *Salmonella (DuPont Qualicon, Wilmington, DE).* The method is a PCR-based rapid, automated method that is highly sensitive. The method is an AOAC approved method and has also been adopted by the USDA/FSIS as a standard method of detecting *Salmonella* in swabs collected from fresh beef and produce. The *BAX® System* detection limit is 10^4 cfu/mL in enriched samples. For *Salmonella*, samples were pre-enriched in Tryptic Soy Broth (TSB) and incubated at 37 C for 24 h. All samples were subjected to standard BAX protocols as described in the BAX guidebook for the presence of *Salmonella*.

Isolation of *Salmonella* from positive samples

All positive samples identified by the *BAX® System* were selectively enriched in Rappaport Vassiliadis (RV) broth and Tetrathionate (TT) broth and incubated at 42° C for 24 h. *Salmonella* isolates were later obtained on Xylose-Lysine-Tergitol 4 (XLT4) agar plates after streaking the plates with a loop from the RV and TT broth and incubating at 40-48 h at 37° C. Confirmation of presumptive positive *Salmonella* isolates was performed by agglutination using Oxoid's Salmonella Agglutination kits. Not all samples testing positive by the *BAX® System* could be recovered for isolation.

Serotyping

All isolates analyzed by the agglutination test and the *BAX® System*, were confirmed as *Salmonella* and were sent for serotyping to the *Salmonella* Reference Center at the University of Pennsylvania. The isolated strains were prepared and sent as follows: the frozen samples were inoculated in TSB broth with 10% glycerol and allowed to grow for 24 h at 37°C, and then 1 mL aliquot was taken out of each sample and put on centrifuge tubes. Three replicates were made per positive sample. All sample replicates were frozen at -80°C. Frozen isolates where then regrown in TSA slants. Once the samples were ready to ship, they were packed safely into an insulated box with ice packs and sent by FedEx overnight. The samples were delivered in approximately 24 h.

Power Test

At the beginning of the study, a power test was conducted to determine sample size. Desired number of samples varies depending on whether the desired outcome is detection of the candidate organism, estimation of the prevalence of the organism within a desired level of precision, or comparison of two groups. While these outcomes all deal with a relatively simple concept, the primacy of specific objectives will dictate very different sample sizes. Without prior information, informed sample size calculation, which was unknown, regardless of desired objective, results in estimation with imprecision. Fiscal limitations were also considered in sample size acquisition.

With limited a priori knowledge of bacterial prevalence, it was determined that 400 samples of each cohort (Country/type of sample - beef vs. produce) would be sufficient. This is based on the fact that the prevalence of *Salmonella* is 5% in type of sample, this sample size proved:

- Precision of 3% for point estimate calculation.
- Probability of failing to detect a positive product of 0.003%.

Statistical Analysis

Data was entered into an electronic spreadsheet and a sample was considered positive if *Salmonella* was detected positive by the *BAX® System*. Data was then imported into a commercially available software package for exploration and analyses (SAS V 9.2, SAS Institute Inc., Cary, N.C). Data were considered to be binomial and analyzed using logistic regression.

Results and Discussion

Salmonella Prevalence in Beef in Honduras

The overall prevalence of *Salmonella*-positive samples in Honduras retail beef (N=393) was 5.9% with a 95% CI [3.9, 8.7].

Table 3.5 shows the overall *Salmonella* prevalence by season. Spring season includes the samples taken in April 2012, summer includes the samples taken in June 2011, fall includes the samples taken in November 2011, and winter includes samples taken in February 2011. The percent positive per season was summer with 9.0% positive (9/100), spring with 7.2% positive (6/83), fall with 4.7% positive (7/147), and winter with 1.6% positive (1/63) (Table 3.5). Statistically there was no seasonality trend, but the summer presented the highest *Salmonella*-positive samples.

Table 3.5 *Salmonella* overall prevalence in retail beef samples by season in Honduras.

SEASON	TOTAL POSITIVE SAMPLES	OVERALL % POSITIVE	TOTAL N SAMPLES COLLECTE BY SEASON
Winter	1	1.6	63
Spring	6	7.2	83
Summer	9	9.0	100
Fall	7	4.7	147
Total	23	5.9	N = 393

Honduras does not have four well-defined seasons, as observed in the U.S. or Europe. Honduras is hot and humid almost year-round. Temperatures vary by altitude rather than season. Overall, this is a tropical climate with temperatures ranging between an average high of 32°C (90°F) and an average low of 20°C (68°F). Rain falls year round in the Caribbean lowlands but is seasonal throughout the rest of the country. Nearer San Pedro Sula, amounts are slightly less from November to April, but each month still has considerable precipitation. The interior highlands and Pacific lowlands were Choluteca is located, have a dry season, known locally as "summer," from November to April. Almost all the rain in these regions falls during the "winter," from May to September. Total yearly amounts depend on surrounding topography; Tegucigalpa, in a sheltered valley, averages only 1,000 millimeters of precipitation. The highest *Salmonella* prevalence in retail beef was observed in June, which is part of the rainy season. The second highest prevalence was observed in April, which is part of the dry season. Climate appears to not be a factor in the epidemiology of *Salmonella* in Honduras. There is no doubt that other factors must play a role in the epidemiology of *Salmonella*, such as diet, management of the animals, cross contamination, vectors, among others.

In Honduras there is not enough information on food-borne prevalence in different food commodities such as food from animal origin or produce. This baseline can be used as a starting point to improve Honduras foodborne surveillance system; as well as help food processors improve their food safety programs, and help them address pre-harvest and post-harvest interventions in the beef industry.

Comparing the results of this study with those presented by Pond et al. in 2010, there is a reported higher *Salmonella* prevalence in Mexican retail beef than in Honduras retail beef. Pond et al. reported a prevalence as high as 33.3% (2/13) in retail beef in the city of Guadalajara, and 12.9% (4/31) in the Mexico City *(63)*. This difference between the two countries is a clear example of how food safety regulations in two different countries can affect the prevalence of foodborne pathogens like *Salmonella*. Further research is needed in order to establish the factors affecting *Salmonella* prevalence in Latin American countries like Mexico and Honduras.

Tegucigalpa had the highest percent positive with 7.7% positive (18/235). Siguatepeque had the second highest percent positive with 4.3% positive (2/47). Choluteca had the third highest percent positive with 4.2% positive (2/48). San Pedro Sula had the lowest percent positive with 1.6% positive (1/63) (Figure 3.1).

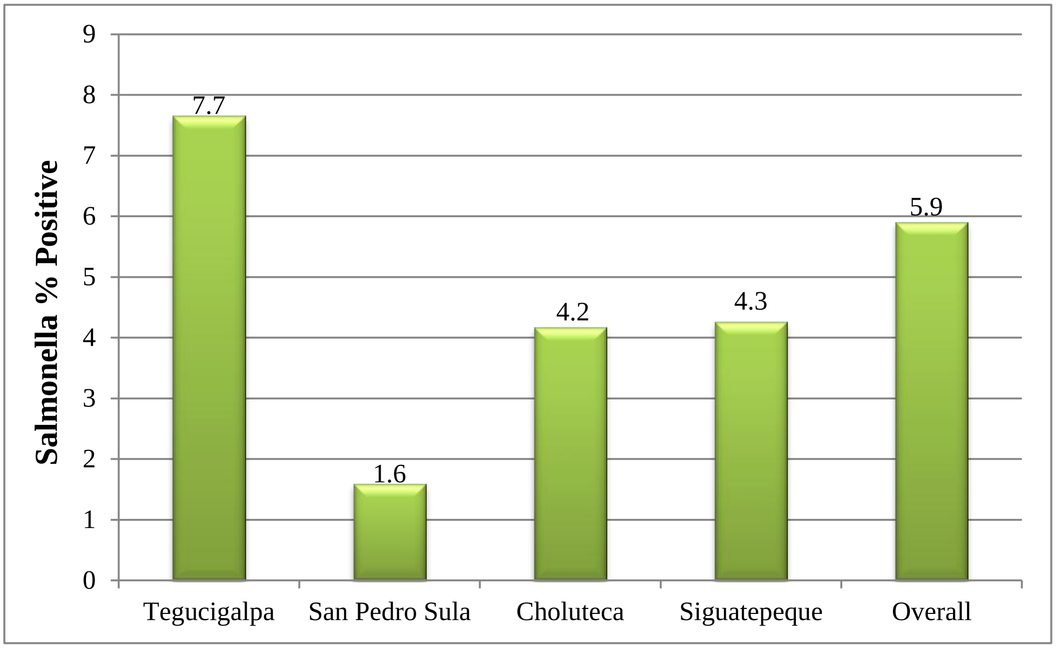

Figure 3.1Overall *Salmonella* prevalence in Honduran beef samples obtained per city (Tegucigalpa, San Pedro Sula, Choluteca, and Siguatepeque) expressed as a percentage of positive samples per total number of samples collected (N = 393). The sampling period includes February, June, November, 2011 and April, 2012.

Salmonella Prevalence in Beef Plants in Honduras

The overall prevalence of *Salmonella*-positive carcass swabs was 7.8% positive in both beef plants in Honduras (11/141). Plant A had the highest percent positive with 10% positive (9/90). Hides samples had the highest percent positive, 23.3% positive (7/30). Pre-evisceration samples had the second highest percent positive with 6.7% positive (2/30). There was a 0.0% in post-evisceration samples (Figure 3.2).

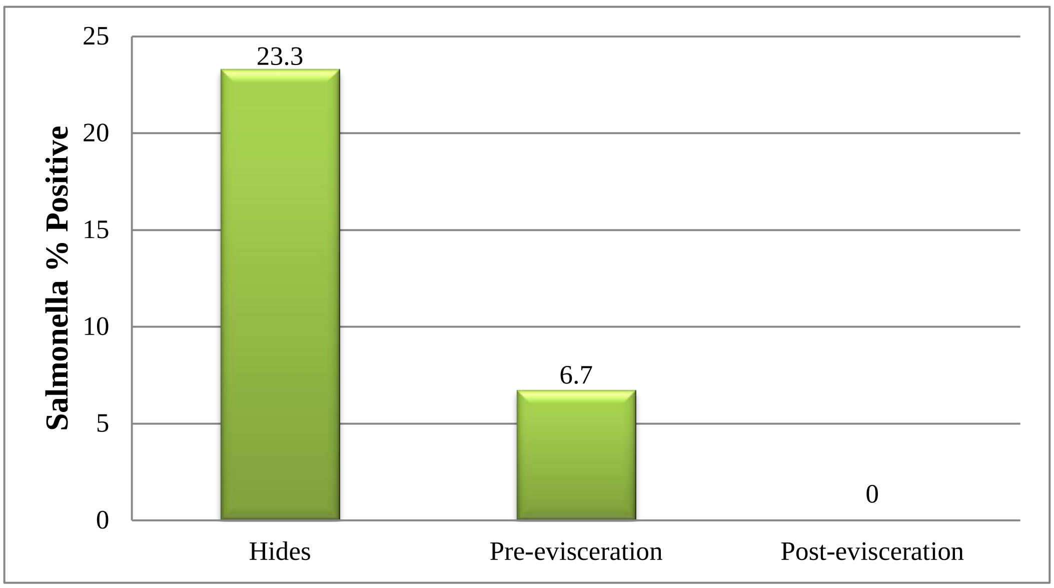

Figure 3.2 *Salmonella* prevalence on beef carcass samples obtained in Plant A (located in Catacamas, Honduras) expressed as a percentage of positive samples per total number of samples collected (N = 90). The sampling period was during April 2012.

Plant B had the lowest percent positive of both plants with a 3.9% positive (2/51). Hides samples had the highest percent positive, 11.8% positive (2/17). There was a 0.0% positive (0/17) in both pre-evisceration samples and post-evisceration samples (Figure 3.3).

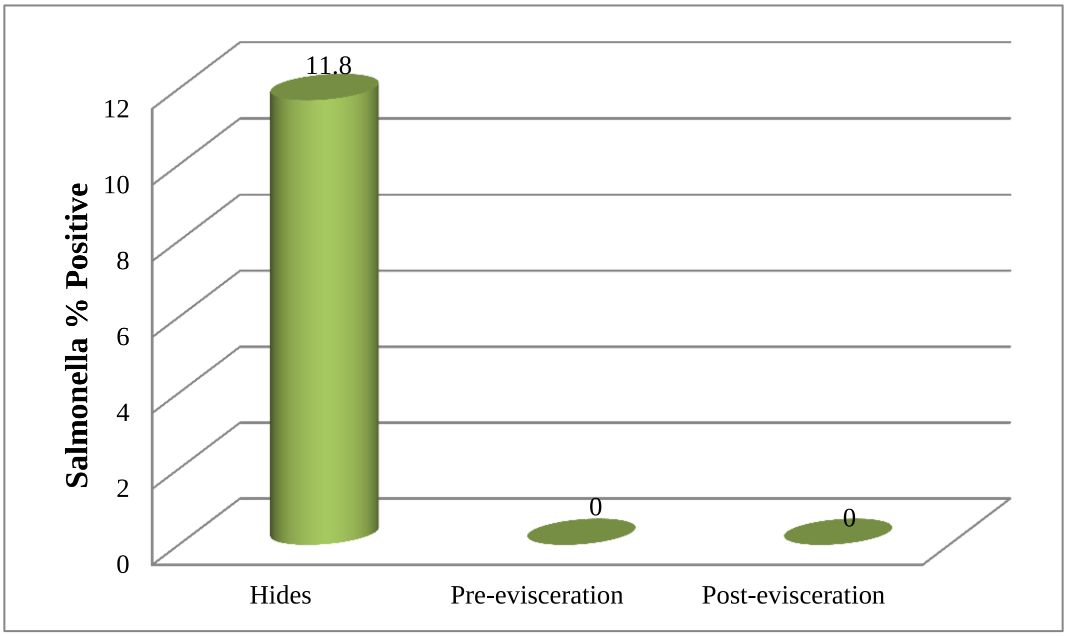

Figure 3.3 *Salmonella* prevalence on beef carcass samples obtained in Plant B (located in Siguatepeque, Honduras) expressed as a percentage of positive samples per total number of samples collected (N = 51). The sampling period was during April 2012.

No *Salmonella*-positive sample was detected on any of the post evisceration samples in both plants. This could mean that the lactic acid interventions used by both beef plants are working on eliminating *Salmonella* from the beef carcass. As mentioned before, Honduras' beef exports are expected to increase in the next couple of years, so it will be interesting to see if this zero detection at post-evisceration carcasses continues as the markets expands. Continuing with this baseline will allow the monitoring of this expansion in beef exports, and if this low prevalence of *Salmonella* continues it will allow Honduras to open and expand its beef markets not only to the U.S., but to other countries worldwide. This baseline can be further used to assess the effectiveness of the Honduran beef industry processes and there compliance with sanitation standards and operating procedures (SSOP), GMP and HACCP. Honduras' Meat Inspection System could eventually be used as an example by other countries, to improve their meat safety programs.

The percent positive on hides (23.3%) on plant A and plant B (11.7%) is relatively low compared to other studies were the prevalence of *Salmonella* on hides was much higher than on our study *(32, 33)*. One clear example is the research done in Mexico by Bravo et al. where the *Salmonella* prevalence on carcass samples was much higher with a 92.0% positive on beef carcass hides in Mexico *(25)*. There are also other studies that have reported a lower prevalence on hides, (37.7%); and (68%) than the one by Bravo et al *(32, 33)*. The low prevalence of *Salmonella* on Honduras carcass samples opens the question of whether this result is a onetime event, or do Honduras really has a lower prevalence of *Salmonella* in beef.

When we compare the Meat Inspection System of Honduras and Mexico for example, there is one major difference between them. In Honduras, all beef is produced under one production system. All the municipal and private slaughter plants are certified by SENASA. Every beef plant, independently of whether they are municipal or private, have to comply with SENASA standards and all of them have a SENASA inspector assigned to them full time to supervise the compliance of these standards. SENASA inspectors have the authority to suspend and delist any certified establishments if they are not in compliance with the meat legislations and standards *(3)*. Also all beef plants in Honduras, regardless of whether they are municipal or private, have to have a HACPP system implemented on their process in order to be in compliance with SENASA standards. In Mexico, on the contrary, beef is produced under two types of production systems. The two production systems include those who comply with the Federal Inspection Certification (TIF) and those who comply with the SAGARPA regulations. Federal Inspection Type slaughterhouses comply with all national and international food safety specifications. Municipal slaughter houses don't have to comply with TIF standards, and the majority of the beef produced in Mexico comes from Municipal slaughter houses *(25)*. HACCP systems are not required and implemented by the domestic market in Mexico, unless the slaughter plant intends to export *(25)*. A comparison between Mexico and Honduras Meat's Inspection System show clears differences between both countries. Honduras beef production works under one whole umbrella of regulations, while Mexico's beef production system works under two umbrellas. It is important to also take into account the difference in size between both countries beef production systems and how food safety culture influences this. Mexico has almost 15 times the population of Honduras, which means they have to provide beef 15 times more than Honduras. If Honduras beef production system continues to expand, it will be interesting to see if there beef production system continues to be as effective in controlling foodborne pathogens like *Salmonella*, as it appears to do so in this study. So far it is clear that Honduras beef production system is working on controlling *Salmonella*, while Mexico has a reported higher prevalence of *Salmonella* which can show the gaps in controlling *Salmonella* between its two production systems *(25, 63)*.

Salmonella Serotypes in Beef in Honduras

A total of 14 isolates have been obtained from Honduran retail beef. The most common serotypes identified in Honduras were *Salmonella* serotype Typhimurium followed by *Salmonella* serotype Derby. This is consisting with studies that have reported an increase in the distribution of *Salmonella* serotype Typhimurium in Latin American countries *(39)*. Also *Salmonella* serotype Derby is among the 20 most frequent serotypes human *Salmonella* isolates in Latin America *(39)*. In the near future our goal is to determine the antibiotic susceptibilities of these frequent human *Salmonella* isolates from Honduras and all serotypes will be further analyzed by pulsed-field gel electrophoresis (PFGE). The purpose of this is to create a more comprehensive baseline of the burden of nontyphoidal *Salmonella* in developing countries like Honduras.

Salmonella Prevalence in Produce in Honduras

The overall prevalence of *Salmonella* in Honduras produce (N=383) was 2.4% with a 95% CI [1.2, 4.5]. No *Salmonella* was detected on any produce type in the cities of Choluteca and Siguatepeque; all *Salmonella*-positive samples were detected in the city of

Tegucigalpa. Cantaloupes had the highest percent positive in Tegucigalpa with 4.8% positive (1/21). Cucumbers had the second highest percent positive with 4.4% positive (2/46). Tomatoes had the third highest percent positive with 3.6% positive (2/55). Peppers had the lowest percent positive with 1.4% positive (1/73). Leafy greens had the second lowest percent positive with 1.8% positive (1/55). Cilantro had the third lowest percent positive with 2.4% positive (1/41) (Figure 3.4).

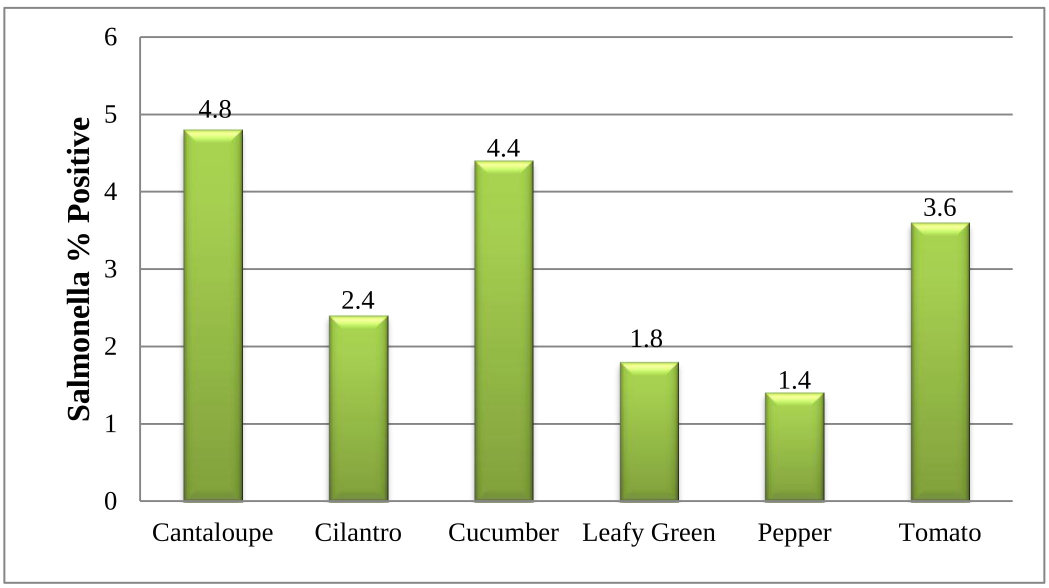

Figure 3.4 Overall *Salmonella* prevalence on produce type (cantaloupe, cilantro, cucumber, leafy green, pepper, and tomato) in Tegucigalpa, Honduras expressed as a percentage of positive samples per total number of samples collected (N = 291). The sampling period includes June and November 2011, and April 2012.

Table 3.6 shows the overall *Salmonella* prevalence by season in Tegucigalpa. Spring season includes samples taken in April 2012, summer includes samples taken in June 2011, and fall includes samples taken in November 2011.

Table 3.6 *Salmonella* overall prevalence in produce samples by season in Tegucigalpa, Honduras.

SEASON	TOTAL POSITIVE SAMPLES	OVERALL % POSITIVE	TOTAL N SAMPLES COLLECTE BY SEASON
Spring	4	2.6	155
Summer	3	3.6	83
Fall	1	1.9	53
Total	8	2.8	291

As with retail beef, the highest *Salmonella* prevalence in produce was observed in June, which is part of the rainy season. The second highest prevalence was observed in April, which is part of the dry season. Again, climate does not appear to be a factor in the epidemiology of *Salmonella* in Honduras.

No *Salmonella* was isolated from Honduran produce for serotyping. This could have several explanations. The amount of *Salmonella* could have been too low to be detectable by cultural methods or the level of background flora could have been too high. Also, the cells could have been injured beyond repair, thus affecting the ability to isolate *Salmonella* using cultural methods even though they tested positive with BAX. The fact that cantaloupes and tomatoes had some of the highest *Salmonella*-positive samples is consistent with studies reporting their high *Salmonella* prevalence and their association with foodborne outbreaks *(18, 27, 38, 49)*.

These results provide reference data that Honduran food processors can use to evaluate their practices and if needed, implement changes at pre-harvest or/and post-harvest levels to improve produce safety. More research is needed in order to establish the risk factors that may contribute to the spread of this pathogen, thus affecting *Salmonella* prevalence in Honduran produce.

***Salmonella* Prevalence in Produce in Mexico**

The overall prevalence of *Salmonella*-positive samples in Mexico (N=514) was 2.1% with a 95% CI [1.2, 3.8]. Veracruz was the city with the highest percent positive with 2.1% positive (3/144). Merida had the second highest percent positive with 1.8%

positive (4/19). Cancun had the lowest percent positive with 1.6% positive (2/126). In Mexico City no *Salmonella*-positive sample was detected on any produce type (Figure 3.5).

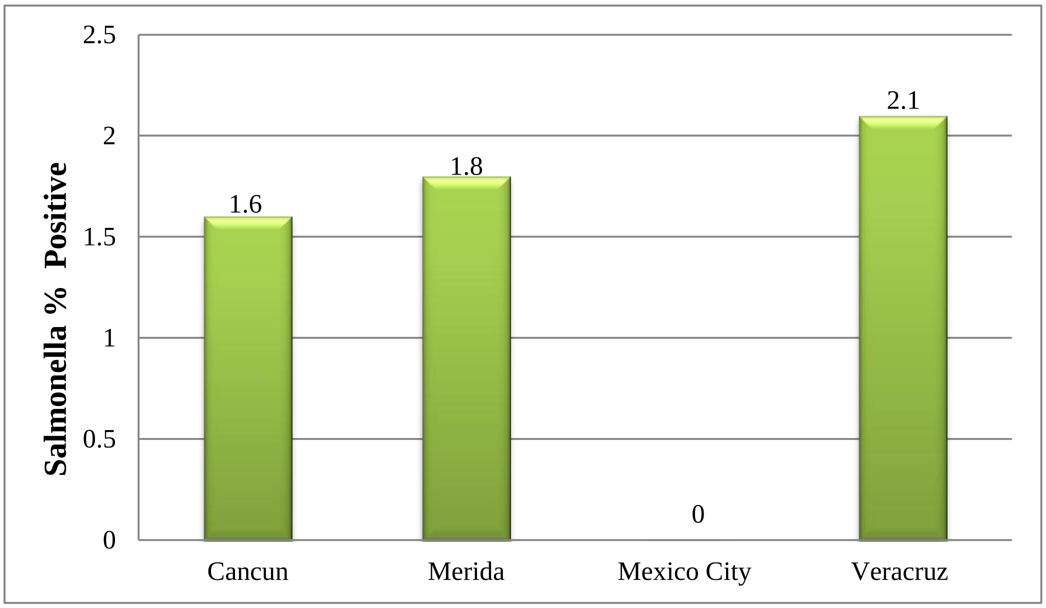

Figure 3.5 Overall *Salmonella* Prevalence on produce samples in Mexico by City (Cancun, Merida, Mexico City, and Veracruz) expressed as a percentage of positive samples per total number of samples collected (N = 514). The sampling period includes April, June, August, September, and December 2011 and April 2012.

In Cancun, cantaloupes were the only produce type that had *Salmonella*-positive samples detected with an 11.8% positive (2/17) (Figure 3.6).

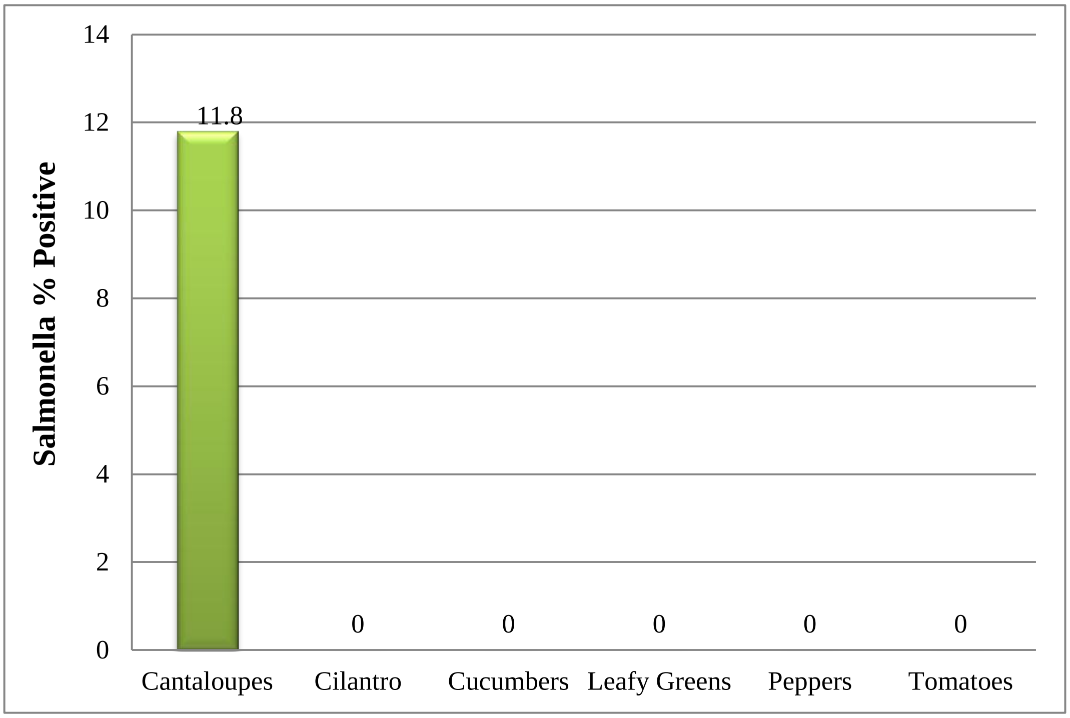

Figure 3.6 *Salmonella* prevalence in Cancun, Mexico by produce type (cantaloupe, cilantro, cucumber, leafy greens, peppers, and tomatoes) expressed as a percentage of positive samples per total number of samples collected (N = 126). The sampling period includes April, August, and December 2011.

In Merida, *Salmonella*-positive samples were only detected in cantaloupes, cilantro, and leafy greens. Positive results were 5.1% positive (2/39) in leafy greens, 3.1% positive (1/32) in cantaloupes, and 2.4% positive (1/41) in cilantro (Figure 3.7).

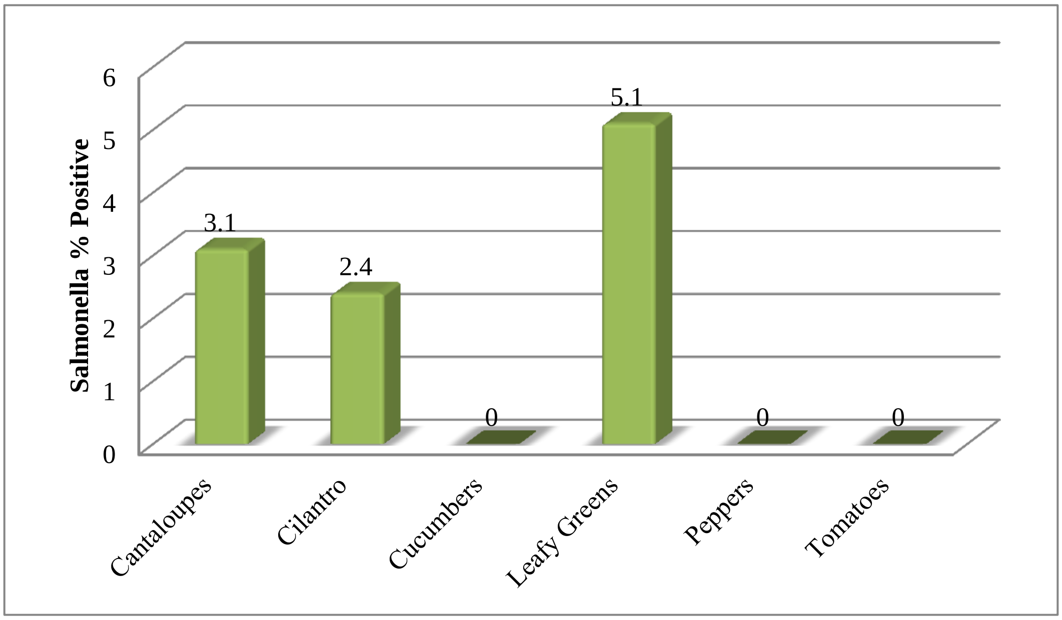

Figure 3.7 *Salmonella* prevalence in Merida, Mexico by produce type (cantaloupe, cilantro, cucumber, leafy greens, peppers, and tomatoes) expressed as a percentage of positive samples per total number of samples collected (N = 219). The sampling period includes April, August, and December 2011 and March 2012.

In Veracruz, *Salmonella*-positive samples were only detected in cantaloupes, leafy greens, peppers, and tomatoes. Positive results were 8.3% positive (2/24) in cantaloupes, 4.0% positive (1/25) in peppers, and 3.9% positive (1/26) in both leafy greens and tomatoes (Figure 3.8). Cucumber was the only produce type that did not have a *Salmonella*-positive sample in all cities in Mexico.

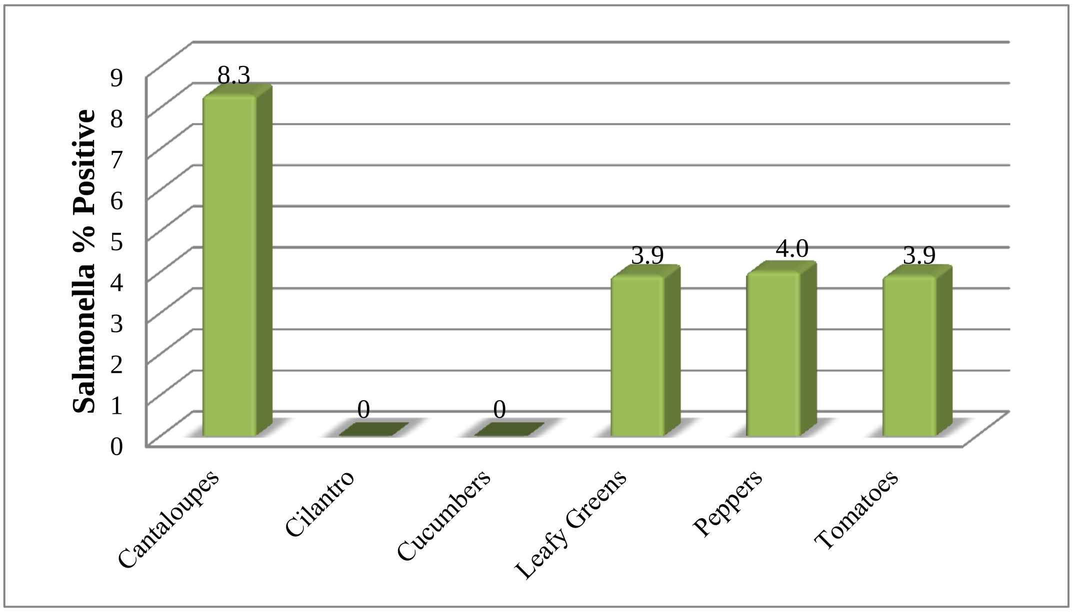

Figure 3.8 *Salmonella* prevalence in Veracruz, Mexico by produce type (cantaloupe, cilantro, cucumber, leafy greens, peppers, and tomatoes) expressed as a percentage of positive samples per total number of samples collected (N = 144). The sampling period includes April, June, and September 2011.

Mexico does not have four well-defined seasons, as observed in the U.S., Canada or Europe. The Tropic of Cancer cuts across Mexico and south of the tropic it's hot and humid all year long along the coastal plains on either side of the country. The hot, wet season runs from May to October with the hottest and wettest months falling between June and September for most of the country. Winter season runs from November to April. On the Yucatan peninsula, where Merida is located, along the mainland Pacific coast, and along the Gulf coast north of Veracruz, annual rainfall reaches 30 inches (80 centimeters) or more. Overall, Mexico has a tropical climate with temperatures ranging between 18°C to 29°C. The highest *Salmonella* prevalence in produce was observed in June, which is part of the rainy season. The second highest prevalence was observed in April, which is part of the dry season. As with Honduras, climate does not appear to be a factor in the epidemiology of *Salmonella* in Mexico. Further research is needed to fully comprehend the factors affecting *Salmonella* prevalence in produce from both Mexico and Honduras.

In Mexico there is also not enough information on food-borne prevalence in different food commodities such as produce or foods of animal original. As mentioned before there have been several outbreaks linked to Mexican produce in the U.S. as a consequence of the poor foodborne surveillance system in Mexico. These outbreaks were found to have originated from produce, such as peppers and tomatoes imported from Mexico. Data collected through a baseline like this study could be used by the Mexican government to monitor changes in the epidemiology of *Salmonella* in Mexico. This information can be essential to determine if existing programs need to be readjusted to ensure that food safety programs are sufficient and appropriate to control *Salmonella* in the Mexican produce industry. Food processors could use this information to implement changes at the pre-harvest or/and post-harvest levels to improve produce safety in Mexico.

Salmonella Serotypes in Produce in Mexico

A total of 10 isolates have been obtained from Mexican produce. The most common serotype identified in Mexico in this study was *Salmonella* serotype Meleagridis. The fact that *Salmonella* serotype Meleagridis is commonly associated with dairy cattle in the U.S., certainly raises questions about the most commonly found *Salmonella* serotypes in Mexico *(20, 46)*. Other *Salmonella* serotypes found in Mexico were Typhimurium, Kentucky, and Newport. These serotypes are consisted with other studies *(38, 39)*. It will be interesting to continue the surveillance of the common *Salmonella* serotypes in Mexico, and see if there is some type of cross-contamination between cattle ranches and produce farms.

CONCLUSIONS

Foodborne *Salmonella* is one of the bacterial foodborne pathogens with highest prevalence worldwide. Although many control measures have been applied over the years to control this pathogen, like lactic acid washes in the beef industry, it still causes great loss to the economy and public health of countries worldwide. *Salmonella* has many transmission routes and reservoirs, making it hard to control its spread. Numerous studies have shown that human salmonellosis affects both developed as well as developing countries. The only thing that varies among countries is the serotypes commonly found. However, health systems in developing countries are not strong enough to help control the spread and burden of this pathogen.

In Honduras the prevalence of *Salmonella* in retail beef found in this study was low compared to other studies conducted in Latin America, like the one conducted by Pond et al in 2010 *(63)*. Another interesting fact about this study was that the low prevalence of *Salmonella* we found in carcass samples in Honduras corresponds to the low prevalence we found in retail beef in Honduras. This fact raises the question of what is the real burden of foodborne *Salmonella* in Central American countries like Honduras, when compared to other Latin American countries like Mexico where there is a reported higher *Salmonella* prevalence in beef carcass and other meat samples *(25, 63)*.

Although the prevalence of *Salmonella* we found in produce in both Honduras and Mexico was low, the produce types like cantaloupes and tomatoes had the majority of positive samples and these are among the produce types commonly associated with food borne outbreaks and this shows that there is greater association between certain produce types and *Salmonella (18, 27, 38, 49).* All the product types sampled in this study are commonly exported to U.S. and other Latin American countries. Fresh produce is a commodity that is eaten raw for the most part, so even a low prevalence can create a serious public health risk when you correlate this to the millions of pounds of fresh produce imported and exported between countries. Also societies have a new mentality of eating healthier, which has in return increased the demand of fresh produce. As a consequence of this increased demand of fresh fruits and vegetables, there has also been an increase in the proportion of food-borne disease outbreaks associated with these food commodities because of the large numbers traded annually between countries *(25).* Despite this low *Salmonella* prevalence found in this study, the research consulted in this study, establishes the importance of *Salmonella* as important etiological agent of diarrhea in both Honduras and Mexico, and it's clear its presence at different points of the food chain.

It is clear that international cooperation is needed to improve food safety worldwide, as well as it is convenient for the participating countries trading different food commodities between each other's borders. Although the prevalence of *Salmonella* was low in this study, *Salmonella* still continues to be a challenge for the food industry worldwide, and a burden for Latin American countries. Creating a baseline study for Latin America will help us get a better approach on the control of foodborne *Salmonella.* This baseline can be used to develop additional criteria and improve food safety standards in the future. It can also help evaluate foodborne pathogens trends like *Salmonella* contamination of beef and produce, and allow food processors to use this information as a tool to improve their food safety systems. This can have a major impact for the international trade, where various food commodities like beef and produce are easily traded between countries.