

SALMONELLA TRANSFER AND SURVIVAL ON TOMATOES AND CONTACT SURFACES UNDER VARIOUS TRANSPORTATION AND STORAGE CONDITIONS

by

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(Under the Direction of Mark A. Harrison)

ABSTRACT

Pathogens can contaminate tomatoes as they contact different surfaces. This study determined *Salmonella* transfer rates between tomatoes and contact surfaces at varying moisture conditions. *Salmonella* inoculated tomatoes were placed in contact with three different food contact surfaces (HDPE, stainless steel, and PVC) while the inoculated surfaces were wet or after areas dried for either 1 or 24 h. Additionally, *Salmonella* inoculated food contact surfaces were placed in contact with uninoculated tomatoes while the inoculated surfaces were wet or after areas dried for either 1 or 24 h. Tomatoes were exposed to contact surfaces for 7 d. Pathogen transfer and survivor rates were evaluated following separation of the tomato and the surface. Wet contact immediately after inoculation resulted in the highest transfer rate. Allowing contaminated tomatoes to dry, prior to contacting surface materials, reduces transfer of *Salmonella*. Maintaining surface contact, over time, promotes survival but has little effect on transfer.

INDEX WORDS: *Salmonella*, tomato, contamination, transfer, cross-contamination

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DEDICATION

To my mother, thank you for inspiring me with your encouragement and strength.

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CHAPTER 1

INTRODUCTION

According to the Centers of Disease Control and Prevention (CDC), *Salmonella* has consistently been one of the leading bacterial causes of foodborne disease in the United States. Tomatoes have served as a vehicle for *Salmonella* during several outbreaks (1-3, 7, 8). For example in 2008, the Food and Drug Administration (FDA) issued a nationwide warning that *Salmonella* was linked to consuming raw tomatoes (5). In order for the industry to meet the demands of their customers, tomatoes and other fresh produce encounter many points of possible pathogen transfer. Since tomatoes are popularly served fresh, there is no final microbial kill step performed prior to consumption.

Trends in the fresh produce industry, such as higher awareness of the nutritional value of fresh produce and year-round demand, have led to an increase in fresh produce consumption (6,7). This increase in consumption of raw product puts a large population at risk in the occurrence of an outbreak. In order to protect the public's safety and provide economic stability, it is necessary to reduce the risk of pathogen contamination.

Salmonella contamination may occur during tomato cultivation, harvesting, handling, packing, and transport (4). Once introduced into the system, *Salmonella* may transfer from a single tomato, to multiple points of contact such as transport containers used in sorting and packing, resulting in contamination of more tomatoes. In order to minimize the spread of *Salmonella* throughout the system and to consumers, Beuchat and Ryu deemed it necessary to thoroughly evaluate produce processing techniques and equipment (4).

By analyzing the steps and environment in which tomatoes are subjected to prior to reaching the public, the industry may be made aware of the steps that leave the product and operations most vulnerable to cross-contamination. The most dangerous points might then be prioritized over taking unnecessary and costly precautionary steps. This study was designed to simulate tomato transportation, and assess the risk of *Salmonella* transfer between tomatoes and surface materials typically seen in tomato packing operations by quantifying cross-contamination.

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Chapter 2

LITERATURE REVIEW

Salmonella

Salmonella are Gram-negative rod bacteria and optimally grow at a neutral pH and at temperatures from 35 to 37°C (40). *Salmonella* growth is inhibited by pH conditions less than 3.8 and greater than 9.0, as well as at temperatures less than 7°C, or at a water activity less than 0.94 (37). Studies have shown that if *Salmonella* has been previously exposed to pH of 5.8, it can adapt and survive at acidic levels as low as pH 3.3 (27). The genetic makeup of *Salmonella* is mainly encoded for pathogenesis and survival (57), and the bacterium can remain viable for weeks in water and years in soil if environmental conditions are favorable (56). Even if the conditions are not favorable, it will adapt to ensure that both pathogenesis and survival are carried out with efficiency, especially survival.

The proficiency of their genetic design allows the pathogen to continuously adapt to its surroundings and enhances the ability to survive. If the conditions, like higher temperatures or lower pH, are not extreme enough to kill the pathogen, the exposure and subsequent adaptation may cause an enhancement in viability, making it harder to completely eliminate the pathogen in question. The genetic variances in virulence throughout the many different serovars create another factor when understanding the mechanisms of its survival and potential for contamination (2).

If ingested, the pathogen must first survive various conditions of the human body, which, other than temperature, are not conducive to *Salmonella* growth. *Salmonella* needs to survive

exposure to an extremely low gastric pH of 1 to 2. The ingested *Salmonella* must then endure the detergents and anoxic nature of the intestines. The microbe needs to travel through the mucin layer of the intestine, in order to reach and invade the intestinal cells (40, 52). As it breaks through the epithelial cells of the villi in the ileum of the small intestine, the pathogen has to make its way into the connective tissue, multiply, and induce endotoxin release (28). If the microbe is capable of remaining viable through such conditions, an incubating period for 5-72 hours within the body will induce a physiological response for the body to emit the invader, by inducing symptoms of salmonellosis which include diarrhea, vomiting, fever, and headache for 1 to 4 days (28). Until the pathogen is completely expelled, the body's immune system will respond by releasing cytokines, a mechanism involved in intestinal inflammation and causes much discomfort to the infected individual (46). In some cases, consuming less than 10 cells is sufficient to cause salmonellosis. Although *Salmonella* generally causes self-limiting gastroenteritis, it may bypass the body's neutrophils and macrophages to find its way into the blood stream, causing the patient to become septic (53). This, of course, depends greatly on the current immune status of the patient in question.

It has been estimated that *Salmonella* causes 35% of the hospitalizations and 28% of deaths caused by foodborne illnesses reported in the United States, a greater health burden than the other foodborne pathogens (16). To prevent such tragic outcomes, studies such as one by Bollaerts et al. in 2008 have been done to determine where the risk factors lie (e.g., minimum dose, susceptible population) that are associated with this pathogen (9). The geographical and seasonal patterns of *Salmonella* outbreaks have even been observed to give the public awareness of when and where they would most likely encounter possible infections (23). Data collected by CDC's FoodNet showed that, for the general population, women were more likely to be infected

during an outbreak. The effects of an outbreak are especially devastating to those with compromised immune systems (21). This population includes but is not limited to patients with heart disease, diabetes, lupus, Crohn's disease, acquired immunodeficiency syndrome (AIDS), the elderly, and infants. When salmonellosis infections occur, where there are several individuals with compromised immune systems, person to person transmission is more likely thus the mortality rate increases (29).

***Salmonella* Outbreaks Related to the Tomato Industry**

Increasing recommended vegetable intake and general healthy eating trends have popularized fruits and vegetables in the American diet (43). While in the 1970s, yearly consumption per capita was calculated at only 149 lbs per year, from 2000 to 2009, the yearly consumption of fresh produce had increased and remained relatively steady at about 196 lbs per year (59). Locations with the capability to grow and distribute fruits and vegetables have greatly benefited economically from a stable demand for fresh produce.

During the 1960s and 1970s, the amount of fresh market tomatoes consumed remained steady at 12.2 pounds per person. A decade later, during the 1980s, fresh market tomato consumption increased by 19%. The rate of tomato consumption went up another 14% during the 1990s to about 17 pounds per person (43). According to per capita disappearance data compiled by the U.S. Department of Agriculture's (USDA) Economic Research Service (ERS), both fresh and processed tomato demand continually grew from 1980 to 2000 (43). Since then, the demand of tomatoes and other fresh produce commodities has been observed to remain high (30). It has been estimated that by 2020, per capita tomato consumption is estimated to remain steady with only a 1.3% increase compared to 2000 (42). With the demand of fresh produce,

particularly tomatoes, making such contributions to the economy, it is necessary to protect this industry from an outbreak that would lead to great losses (52).

Food contamination creates an enormous social and economic burden on communities, growers, manufacturers, distributors, retailers, and health systems. In the late 1960s, *Salmonella* emerged as a major poultry-associated pathogen (6). In 2007, DeWaal and Plunkett expressed concern that consumer confidence in the safety of the food supply waned in response to reoccurring outbreaks of foodborne pathogens (22). After every outbreak, the majority of the public associated that particular product with danger and avoided purchasing that product. In the United States, diseases caused by foodborne pathogens alone were estimated to cost up to \$35 billion annually in medical costs and lost productivity (60).

In 2008, the entire country was warned of an outbreak that linked *Salmonella* to eating raw red plum, red Roma, and round red tomatoes. Months later, during the spring season, tomatoes were technically safe for consumption; however, due to the prior warnings, tomatoes were still perceived negatively by consumers. A general nationwide warning caused distrust throughout the population and damaged the tomato industry. Profit for the tomato industry suffered when they began selling tomatoes at reduced prices or not being able to sell at all because of low demand. The production value losses totaled millions of dollars (24). A decrease in farmer revenue led to decreased expenditures, which further impacted the economy

Due to increased fresh vegetable intake, fresh produce, including tomatoes, has been frequently linked to transmitting pathogens to the consumers. The U.S. Department of Health and Human Services noted that since fewer consumers grow and prepare their own foods, choosing instead to utilize the convenience of supermarkets or to eat in restaurants, these consumers choose to trust the industry in handling products in a safe and sanitary manner (58).

Prior to harvest, *Salmonella* contamination onto fresh produce originates from contact with animal feces; therefore, the contamination is believed to begin at the earlier stages of the supply chain and later spread by distributors, retailers, and finally to consumers (1). *Salmonella* can remain viable after it has been expelled from the gut and is capable of spreading into sewage, soil, and water (50). The number of foodborne outbreak incidences indicates existing flaws within the tomato industry's production and distribution routines. Before reaching the consumer, the product can become contaminated with a foodborne pathogen at some point in the food production, processing, packing, transportation or storage of the tomatoes. When groups of consumers become infected, the industry is made aware of the necessity in improving that system. This is especially important in the fresh produce industry, considering the products cannot undergo a microbial "kill step" prior to packaging and distribution. In a study involving a panel of food safety experts who were surveyed on the risks involved in transporting food, fresh produce was voted to be one of the "high-risk foods across all modes of transportation" (1). In the 1970s, 0.7% of outbreaks from a known food source were from fresh produce. That rate increased to 6%, in the 1990s (55).

During the 1990's, the detection of foodborne outbreaks greatly improved through advancements in surveillance and epidemiological methods. For example, PulseNet is a network that utilizes pulsed-field gel electrophoresis (PFGE) to quickly match strains from patients to the food source (19), thereby being able to alert the public of the outbreak sooner. The CDC deems 2 or more cases of a similar illness which result from eating the same food as a "foodborne outbreak" (12). These outbreaks have been thoroughly investigated in order to protect the health of the public by preventing similar outbreaks from happening in the future. In 2006, the CDC

received reports of a total of 1,270 foodborne disease outbreaks, which resulted in 27,634 cases of illness and 11 deaths (15).

Salmonella has been isolated in a variety of retail foods (61), with products sold raw a greater risk to the public. When a study was done to model the growth of *Salmonella* on tomatoes, it was determined that growth on tomatoes closely resembled growth on poultry (51). Unfortunately, the potential growth creates greater problems for the tomato industry since tomatoes are more often used in their raw state, unlike poultry, leaving existing *Salmonella* viable on the product.

After harvest, *Salmonella*, that has entered the system, can remain viable throughout the whole packing system. Once it is carried into a facility, the pathogen can contaminate the facility and other tomatoes. If contamination goes unnoticed, more and more products become compromised and shipped to the public. In the United States, there have been 12 outbreaks caused by *Salmonella* related to tomato consumption from 1998 to 2005 (10). In 2006, the CDC concluded that contaminated tomatoes were responsible for the outbreak of *Salmonella* Typhimurium (18). In 2008, tomatoes were again under investigation for a nationwide *Salmonella* outbreak (14). The investigation eventually concluded that jalapeño peppers were the major source of contamination; however, tomatoes were still considered a possible source of *Salmonella* early in the outbreak (17).

A multi-state outbreak in the 1990s was determined to have originated from a single tomato packer. The investigators declared that the contamination most likely occurred at the packing shed. Before the field grown tomatoes were packaged, they were all washed in a common water bath. In that water bath, *Salmonella* was believed to have contaminated other tomatoes within the operation (34).

In 2001, Cummings et al. investigated a multi-state outbreak that left 44% of the victims reporting bloody diarrhea, 50% vomiting, and 25% hospitalized. In this study, isolates were collected from the tomato outbreak that occurred between 1998 and 1999. The cause was determined to be restaurant-prepared raw tomatoes, where it was thought that contamination most likely occurred at the farm or during packing. The few pathogen-controlling elements in the commercial tomato packinghouses (e.g., warm, chlorinated wash water) were not enough to eliminate the pathogens; therefore, their conclusion was “More effective disinfection and prevention strategies are needed” (20).

In 2002, tomatoes, grown and packed on the eastern shore of Virginia, were contaminated with *Salmonella* and caused 510 illnesses in 26 states (32). Another multistate outbreak of *Salmonella* infections occurred between 2005 and 2006. Once again, contaminated raw tomatoes were identified as the cause. It was later determined that, for both outbreaks, the tomatoes were grown on two farms on the eastern shore of Virginia. The outbreak strain of *Salmonella* was isolated from irrigation pond water near tomato fields in this region in October 2005 (11).

For *Salmonella* outbreaks that occurred during the summer of 2004, Roma tomatoes were determined to be the vehicles for 561 illnesses (13). Although the CDC’s study of the outbreak concluded that the packinghouses abided by food-safety guidance, only 1 of the 9 inspected facilities was actively operating at the time of inspection. Once again, the cause of a *Salmonella* outbreak from tomatoes was believed to have occurred from cross-contamination during the transporting in and out of a single tomato-packing house (10, 32, 34).

Potential Sources of Contamination

Cross-contamination is defined as “the transfer of harmful bacteria to food from other foods, cutting boards, utensils, etc., if they are not handled properly” (26). The nature of first

growing a commodity in an open field to later distributing it in large quantities to be served without cooking, allows for several points of possible contamination. Keeping in mind consumer acceptability, the industry does not expose fresh produce to conditions that could inhibit pathogen survival, in order to uphold the product's qualities such as texture and color. For example, exposing tomatoes to temperatures, low enough to inhibit pathogen growth, can result in abnormal ripening, pitting, water soaked areas, increased susceptibility to fungal infection, and other forms of chilling injury (49). Maintaining the safety of the product is negated if consumers no longer purchase the product at all; therefore, it is imperative for contamination to be prevented at all points from harvest to distribution.

Naturally occurring throughout the environment, *Salmonella* has many opportunities to be introduced to tomatoes and other fresh produce. It can be distributed in the form of fecal droplets from animals or even from contaminated soil from brought in by strong winds. Although growers have made great attempts to protect their tomatoes from nature's intruders, it is impossible to shield tomatoes from all of them. Growing tomatoes in large open fields leaves them susceptible to pathogen contamination from the environment.

In order to control possible contamination, opportunities for *Salmonella* to enter the chain of distribution have been identified to better educate the industry in proper handling practices (1). Improper use of manure as fertilizer and irrigating with untreated contaminated sewage water have been seen to initially introduce *Salmonella* into the system (8). Commercially available fertilizers, a synthetic formulation of nutrients, can be used instead of manure to support the life of the plant, but if carelessly used, they are more than capable of supporting pathogen life. It has been shown that *Salmonella* can survive in fertilizer, even after diluting the solution. Potentially contaminated fertilizer solutions, often mixed in large batches, are held for

days leaving the pathogen able to multiply to great numbers (47). *Salmonella enterica* can survive for up to six weeks in soil with the ability to contaminate surrounding tomato plants (5). A study done by Hintz et al. determined that irrigation water, containing *Salmonella*, can potentially allow for the pathogen to remain viable in niches found on the tomato, when applied directly to the base of the plant (35).

After inoculating the stems and flowers of tomato plants, Guo et al. showed that *Salmonella* can survive from the time it is inoculated during the flowering stage until the fruit is fully ripened (33). This suggests that *Salmonella* introduced at the earliest stages of harvest may find its way into consumer homes. A study performed by Shi et al. on the persistence and growth of *Salmonella* on pre- and postharvest tomatoes demonstrated a diverse range of *Salmonella* serovars able to establish themselves on the surfaces of preharvest tomatoes, as well as within them. The majority of *Salmonella* can grow and become established both on unripe and ripe tomatoes. The study showed that the amount of growth on ripened fruit depended on the serovar. They claimed that their results provide a possible explanation of why only a select number of *Salmonella* serovars are associated with foodborne illness outbreaks linked to tomatoes (54).

After harvest, transporting tomatoes allows for even more chances of contamination. As the tomatoes travel together in bulk, they touch each other as well as surrounding surface areas used to hold and move each of them. Although routine cleaning and scrubbing throughout packing house facilities is suggested and, in most cases required, typical scrubbing does not suffice in complete pathogen elimination (25). Currently, there is very little information available on the state of food transportation and holding practices in the United States (1). In order to assess points of possible transfer, it is important to note the points where tomatoes are

held in one place for a significant amount of time and to note exposure to vehicles for transfer like water and moving air.

Ackerly et al. stated that during transportation, the greatest food safety concerns are associated with improper holding practices for food products during inspection, and shipping improper management and quality of transportation materials. Along with improper loading practices, conditions, or equipment, fresh produce packing facilities and the products are at risk for cross-contamination (1). The preventative controls for food transportation safety hazards include “appropriate packaging and packing of food products and transportation units”, meaning good quality and correct use of materials (1). Materials of any quality may provide niches for pathogen survival and later transfer. Some surface materials used in packing facilities dry faster and clean easier than others. It was discussed among all surveyed in this report that developing and understanding appropriate procedures for loading and unloading for transportation units is necessary (1).

After *Salmonella* is introduced into a tomato packing facility, it can remain viable in the environment. In order to maintain the quality of the tomatoes, environmental conditions are kept at various temperatures, none of which are high enough to kill the pathogen. The pathogen can stay on the product and cross-contaminate other surfaces in the packing facility. Though these conditions are not the most optimum for pathogen growth, they are more than sufficient for survival. For example, one study showed survival but little *Salmonella* growth has been observed on tomatoes stored at 4, 12, and 21°C (7).

Mahovic et al. recovered *Salmonella* from tomato surfaces and showed that *Salmonella* can survive in the environment of typical ripening rooms (44). Mature green tomatoes are usually held for a number of days at 20 to 21°C in ripening rooms, where they are treated with

gaseous plant growth regulator ethylene (C₂H₄). Typically, they are held for no more than 3 days, but have been observed to have been held under these conditions for as many as 9 days (44). Tomatoes are commercially stored at 75% to 95% relative humidity (7). These claims are supported by studies that recovered *Salmonella* from ripening room conditions [$\approx 20^{\circ}\text{C}$ and 85% to 90% or higher relative humidity] surviving and multiplying for at least 7 days (62).

Tomatoes can provide a favorable environment for growth in spite of low pH values (3). The ability to adapt to acidic pH levels was shown by Leyer and Johnson. Their studies revealed *Salmonella*, previously exposed to hydrochloric acid at pH 5.8, exhibited better survival in acidic foods than *Salmonella* that had not been previously exposed to hydrochloric acid (41). Visible water is another condition believed to aid the spread and survival of *Salmonella*. In environments with a low water activity, *Salmonella* cannot multiply but may be able to survive for days under dry conditions (31).

Salmonella readily adhered to inert food processing surfaces such as the stainless steel and polyethylene used in a study done by Manijeh et al (45). Many bacteria produce exopolysaccharides to form biofilms, which aid them in adhering to plant or inert surfaces from where they can be disseminated (4). It has been shown that the production of biofilm increases *Salmonella* survival on surfaces of fresh produce (39). When *Salmonella* produces biofilm, its survival onto commercially used surfaces under dry conditions is enhanced (36), and points where *Salmonella* survives are all points of great risk for cross-contamination. In 2007, Moore and others observed transfer from industry surface materials (e.g., stainless steel) onto fresh produce (48). Kusumanigrum et al studied the transfer of *Salmonella* from kitchen sponges to stainless steel surfaces and from these surfaces to foods were. *Salmonella* Enteritidis was

recovered from surfaces, after 4 days, indicating a risk for cross contamination from surface to surface (38).

Summary

Although contamination might be prevented by promoting and teaching good agricultural practices (GAP), the ubiquitous occurrence of *Salmonella* provides difficulties in completely removing it from fresh produce. While studies have been done to evaluate *Salmonella*'s survival on surfaces, its rate of transfer has yet to be quantified in order to evaluate the risk of transfer throughout the system. Determining the points of greatest cross-contamination will provide necessary information to educate the industry. Understanding more about the nature of *Salmonella* throughout the fresh produce distribution system may provide information necessary for the industry to alter practices, thereby preventing future outbreaks.

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CHAPTER 3

***SALMONELLA* TRANSFER AND SURVIVAL ON TOMATOES AND CONTACT SURFACES UNDER VARIOUS TRANSPORTATION AND STORAGE CONDITIONS**

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ABSTRACT

During handling and transportation from the field to the market, tomatoes may become contaminated with pathogens as they contact different surfaces. Since water influences pathogen transfer and survival, reducing moisture on tomato surfaces may reduce pathogen transfer. This study determined the rate of *Salmonella* transfer between tomatoes and contact surfaces at varying moisture conditions. Two protocols were conducted. In the first, *Salmonella* inoculated tomatoes were placed in contact with three different food contact surfaces (HDPE, stainless steel, and PVC) while the inoculated surfaces were wet or after the inoculated areas dried for either 1 or 24 hours. In the second, *Salmonella* inoculated food contact surfaces were placed in contact with uninoculated tomatoes while the inoculated surfaces were wet or after the inoculated areas dried for either 1 or 24 hours. Tomatoes were exposed to contact surfaces for 4 time periods at 25°C and 85% humidity: 0 (immediate touch), 1, 4 and 7 days. Pathogen transfer and survivor rates were evaluated following separation of the tomato and the surface by enumerating the *Salmonella* presence on the surfaces. Wet contact immediately after inoculation resulted in the highest transfer rate. Transfer rates were lowest when inoculated tomatoes were dried for 24 h prior to contacting surfaces. Allowing contaminated tomatoes to dry, prior to contacting surface materials, reduces initial *Salmonella* levels. Maintaining surface contact, over time, promotes survival but has little effect on transfer.

INTRODUCTION

In 2010, it was estimated that 40,000 cases of salmonellosis occurred in the United States. (23). *Salmonella* is one of the leading foodborne pathogens and was determined to be the cause of 35% of the cases that resulted in hospitalization and 28% of those that resulted in death (24). Fresh produce products, including tomatoes, have previously served as a vehicle for the transmission of *Salmonella* in many of these outbreaks (4, 5). In order to prevent such outbreaks, it is imperative to identify risk factors for the industry to alter practices that currently allow for *Salmonella* contamination in fresh produce. Since *Salmonella* has been observed to attach to food contact surfaces (34, 52, 69), the possibility of cross-contamination during handling is of concern as items like tomatoes are exposed to these surfaces.

Salmonella has been observed to attach to food contact surfaces (34, 52, 69). Before tomatoes reach consumers, they are picked, sorted, and typically packed in a packinghouse. Once *Salmonella* is introduced into a tomato packing system, this pathogen might transfer to bins, conveyor belts, or the many other surfaces that the tomatoes contact within the operation (16). *Salmonella* remaining on these surfaces can further contaminate other tomatoes entering the system, and those contaminated tomatoes can contaminate more surfaces throughout the operation. This cycle of cross-contamination from surface material to tomato and tomato to surface material can allow for foodborne pathogens to spread throughout the public quickly. Identifying the conditions that most contribute to *Salmonella* transfer from surface to surface, as well its survival once transferred, can aid the tomato industry in preventing the cross-contamination of *Salmonella*.

In this study, the risk of cross-contamination was measured by calculating transfer coefficients for *Salmonella* under environmental conditions that could occur within a commercial

setting. These conditions may enhance the risk for *Salmonella* to survive and cross-contaminate. Since water may enter the system at several points and at different levels within a facility, varying levels of available water were compared to determine this effect on pathogen transfer. Holding the pathogen between surfaces may promote its survival; therefore, the amount of time a tomato may remain in contact with a food contact surface material depends on its stage in the operation. Determining the points of greatest cross-contamination will improve tomato handling practices and may prevent reduce the spread of *Salmonella* onto their products and to their consumers.

MATERIALS AND METHODS

Preparation of inoculum and growth media

Salmonella enterica serovars Michigan MDD251 (cantaloupe outbreak), Montevideo MDD236 (tomato outbreak), Newport MDD314 (tomato outbreak), Poona MDD237 (cantaloupe outbreak), and Saintpaul were maintained at -80°C beads with a cryoperservative (Microbank, Pro-Lab Diagnostics, Austin TX). The strains were provided by the Citrus Research and Education Center, University of Florida, Lake Alfred, FL and were adapted for resistance to 100 µg/ml of rifampicin.

To prepare the inoculum for each experiment, each strain was grown separately, in 10 ml of tryptic soy broth (TSB; Becton Dickinson and Company (BD), Sparks, MD) containing, 20 µl of a 5% solution of rifampicin (100 µg/ml; cat. # BP267925; Fisher Scientific, Pittsburgh, PA). The rifampicin was prepared by slowly dissolving 1 g of rifampicin in 20 ml of methanol (Fisher Scientific) before filtering through a 0.22 µm sterile cellulose acetate bottle top filter (Fisher Scientific). The solution was protected from the degrading effects of light and stored at 4°C. Inoculated broth tubes were incubated at 37°C for approximately 24 h. Each tube was

centrifuged (Beckman Coulter, Allegra TM X-22R Centrifuge, Fullerton, CA) for 10 min at approximately 1,700 x g at 4°C. The supernatant was discarded and the remaining pellet was washed twice with 10 ml of phosphate buffered saline (PBS; BD). After the second wash, 5 ml of 0.1% peptone (BD) water was used to resuspend each pellet prior to combining them in equal volumes to form the pooled inoculum.

Tryptic soy agar with 100 µg/ml rifampicin (rifampicin added to tempered agar; TSAR; BD) was used to enumerate *Salmonella*.

Tomatoes and food contact surfaces

Mature green tomatoes were purchased from a local retail grocer and stored at 4°C for no longer than 3 d prior to inoculation. Each food contact surface consisted of a tomato/surface material pair. Each tomato was paired with a surface chosen to mimic surfaces used in tomato packing and transportation operations. High density polyethylene (HDPE, 0.08 cm thick, United States Plastic Corporation, Lima, OH), stainless steel (type 304, 4B finish), and white polyvinyl chloride sheet (PVC; 0.3 cm thick, United States Plastic Corporation) were chosen to simulate contact surfaces that tomatoes commonly touch during transportation and distribution. The surfaces were cut into 8 cm x 8 cm squares.

Inoculation of tomatoes and food contact surfaces

A marked area (~3 cm²) on the flattest side of each tomato and the center of each food contact surface was spot inoculated with 0.1 ml of the *Salmonella* cocktail. Two experimental protocols were conducted. In the first, tomatoes inoculated with *Salmonella* were placed in contact with three different food contact surfaces while the inoculated surfaces were wet or after the inoculated areas dried for either 1 or 24 h at room temperature. In the second protocol, each food contact surface inoculated with *Salmonella* was placed in contact with an uninoculated

tomato while the inoculated surface was wet or after the inoculated area dried for either 1 or 24 h at room temperature. Tomatoes were then exposed to contact surfaces commonly found in produce transportation containers, maturing rooms, and repacking facilities (HDPE, stainless steel, and vinyl) for four time periods at 25°C and 85% humidity: 0 (immediate touch), 1, 4 and 7 days. Pathogen transfer was evaluated following separation of the tomato and the surface by rinsing both with 20 mL of 0.1% peptone, and spiral-plating onto TSAR. TSAR plates were incubated at 37°C and surviving *Salmonella* enumerated after 24 h. Controls included tomatoes inoculated and allowed to dry as described, but with no exposure to surfaces and contact surfaces inoculated and dried as described, but with no exposure to tomatoes.

Recovery of *Salmonella* from tomatoes and food contact surfaces

At designated sampling times, tomatoes were placed in sterile bags with 20 ml of 0.1% peptone and manually massaged for 30 s and shaken for 30 s with the inoculated areas in contact with the peptone water at all times. The HDPE and vinyl samples were placed in sterile stomacher bags with 20 ml of 0.1% peptone water, which was stomached (Seward, 400 Circulator, UK) for 90 s. To avoid puncturing the bags with sharp corners, stainless steel pieces were placed in sterile stomacher bags, 20 ml of 0.1% peptone water was added, and the pieces were held flat side up, vigorously shaken for 45 s, flipped over, and shaken for 45 s. Samples were serially diluted in 0.1% peptone and spiral plated (Spiral Biotech, Autoplater 4000, Bethesda, MD) onto TSAR. All plates were incubated for 24 h at 37°C and colonies were enumerated. Transfer coefficients were calculated by dividing either the number of *Salmonella* transferred to the food contact surfaces from the inoculated tomatoes by the number of *Salmonella* initially inoculated onto the tomatoes or by dividing the number of *Salmonella* transferred to the tomatoes from the inoculated contact surfaces by the initial number of

Salmonella initially inoculated onto the food contact surfaces.

Statistical Analysis

Three replications of each treatment were performed and nine samples were analyzed for each treatment combination for each replication. To compare *Salmonella* survival for the inoculum conditions, each count was analyzed after log transformation. The significant effects of evaluated treatments were analyzed through two-way ANOVA in Minitab. The level of significance of this study was $p < 0.05$. When significant effects were seen in ANOVA, Tukey's multiple comparison method was used to determine the difference among means.

RESULTS

In this study, cross-contamination of *Salmonella* between tomatoes and food contact surfaces and vice versa was quantified by evaluating the number of *Salmonella* transferred between the surfaces. Survival of *Salmonella* on all of the surfaces over a period of time that might be encountered in tomato distribution was also determined under different levels of dryness. Three different food contact surfaces were used in this study (HDPE, stainless steel, and polyvinyl chloride); however, since the 3 surfaces are not interchangeable for their use within the industry, the surfaces were not compared. A transfer coefficient of less than 1.00 indicated *Salmonella* was observed to have a higher affinity for the initially inoculated surface.

When either the tomatoes or food contact surfaces were initially inoculated with approximately 5 logs cfu of *Salmonella*/cm² and remained wet prior to exposure to new surfaces, a transfer coefficient for day 0 and after 1 day of continuous contact was between 0.80-1.20 (Tables 3.1-3.3). This indicates that, when not dried prior to contact to a clean surface, *Salmonella* will transfer as easily as it can remain on the initial surface regardless of whether the tomato or the food contact surface was originally contaminated. During wet contact at day 0, the

inoculum droplets were visible and easily transferred onto the uninoculated surface area. Even though the tomatoes and food contact surfaces remained in direct contact over a 7 day storage period and the wet inoculated areas dried during storage, the transfer coefficients calculated for days 4, and 7 for the tomatoes and surfaces in contact over time were similar to that for days 0 and 1.

After allowing either the tomatoes or food contact surfaces to dry for 1 h prior to exposure to new surfaces, transfer coefficients were generally lower than wet exposure. Transfer between polyvinyl chloride and tomatoes, when tomatoes and food contact surfaces were held in contact over time after the 1 h drying period, was between 0.63 and 1.12. Transfer from tomatoes to HDPE (Table 3.1) dropped from 1.06 to 0.47 after 1 day of exposure to the inoculated tomato. Transfer coefficients from tomatoes to stainless steel also decreased over time, from 1.10, after day 1, to 0.56, after day 4 (Table 3.2).

Allowing a contaminated surface to dry for 24 h prior to exposure to a clean surface showed the least risk for pathogen transfer. After allowing either the tomatoes or food contact surfaces to dry for 24 h prior to exposure to the clean surfaces, transfer coefficients were significantly lower than when the inoculum was wet or after 1 h of drying ($p < 0.05$). The transfer coefficients were between 0.03 and 0.58. This indicates that, when dried prior to contact to a clean surface, *Salmonella* will not transfer as readily to a new surface regardless of whether the tomato or the food contact surface was originally contaminated.

Regardless of which surface was initially inoculated drying the inoculated surfaces prior to contact decreased initial *Salmonella* populations on both surfaces (Figures 3.1-3.6). Drying for 24 h showed a significant reduction in pathogen survival in comparison to both the wet and 1 h dry ($p < 0.05$), while *Salmonella* survived best when the inoculum remained wet or damp. Even

when the inoculum was only given 1 h to dry prior to contacting new surfaces, the *Salmonella* populations remaining on the surfaces were significantly affected ($p < 0.05$) compared to both of the other treatments.

DISCUSSION

Before tomatoes reach consumers, several possible risk points for *Salmonella* cross-contamination are encountered. While in the field, tomatoes are susceptible to fecal contamination by animals. The pathogens encountered from the field may survive throughout packing and transportation and can contaminate packinghouse materials (shipping containers, conveyor belts, etc.) to further contaminate additional tomatoes. Typically fresh produce is not exposed to conditions that would kill pathogens, so the consumer receives the products raw; therefore, studies must be done to determine where the greatest risk for pathogen transfer and survival exist in order to efficiently prevent an outbreak.

This study was done to evaluate the risk of cross contamination between tomatoes and surface materials used in commercial packing operations. From harvest to packaging, water can be introduced to the tomato transportation system at multiple points and at varying levels. In a typical commercial operation, visible water droplets on the tomatoes can be collected from the dew. These droplets may dry to various levels prior to reaching the packinghouse. Once in the packinghouse, the tomatoes are usually dumped in soak tanks for washing. Although the product can be dried by either air blowers or brushes (50), the level of moisture remaining on the product varies. Water droplets can carry *Salmonella* throughout the operation.

In this study, *Salmonella* was inoculated onto either tomatoes or food contact surfaces and held under three conditions (wet, 1 h dry, 24 h dry) prior to contacting uninoculated surfaces to simulate the possible moisture levels on tomatoes or materials used in packinghouses and to

evaluate the potential for cross contamination. While water can act as a carrier for *Salmonella*, it is still capable of remaining viable under dry conditions (13, 49, 54, 71). In this study, samples contacted immediately after inoculation were “wet”. Greater numbers of *Salmonella* were transferred between the surfaces that were wet than those dried prior to contact. Drying did not completely eliminate the pathogen.

Previous research has been done to examine the conditions typically found throughout a tomato harvesting and packing operation, which are taken to maintain the highest quality of product. Many studies have addressed consumers’ concern with quality (78, 79, 91). For example, one study determined that exposing green tomatoes to longer times at chilling temperatures resulted in “reduced marketable life, dull color, flaccidity, and delayed, uneven (blotchy) and nonuniform ripening”. Firmness was lost over time as when exposed to chilling temperatures (28, 73).

Based on shipping trials observed in a study done by Dea et al. (32), the holding temperature of received tomatoes was 25°C. In order to simulate the conditions similar to those tomatoes experience during shipping and maturation, studies were conducted at 25°C to observe *Salmonella* survival. As seen in Figures 3.1-3.6, *Salmonella* survived for up to 7 days. Since over time there were no significant changes in *Salmonella* levels ($p>0.05$) when samples were held in contact to each other, it can be concluded that holding *Salmonella* in between surfaces may provide protection to enhance its survival. Previous studies have shown that *Salmonella* populations on tomato surfaces decrease over time. In one study done to evaluate *Salmonella* survival over time, *Salmonella* populations on tomato surfaces at various temperatures decreased over a 28 day period (3). In another study by Guo et al., the number of cells inoculated on tomatoes decreased by approximately 4 log cfu/tomato during storage for 14 days at 20°C (46).

In that study, *Salmonella* survival on tomatoes in contact with soil was observed. The fruits in contact with inoculated soil at 20°C harbored *Salmonella*, where the population of *Salmonella* on tomatoes in contact with soil increased.

Under the conditions studied, transfer coefficients were calculated to determine the highest risk for cross contamination. The highest coefficients were calculated when the surfaces were contacted immediately after inoculation and sampled on the same day. When contacting the two surfaces immediately after inoculation, no time was given for the pathogen to attach to the initial surface; therefore, most of the inoculum was displaced onto the second surface area making the difference between the two greater than the treatments that were contacted over time.

Although the 24 h dry treatments were typically observed to be less favorable for *Salmonella* survival and transfer (Figures 3.1-3.6), after being held in contact for 7 d, an increase in enumerated *Salmonella* was observed. This could be explained by the qualitative changes in the product. Over time, in the 25°C incubator, some tomatoes showed signs of rot and visible mold. It has been shown that damaged tomatoes support *Salmonella* viability (10). Although the tomatoes were handled carefully in this study, the handling of the tomatoes prior to their arrival could not be controlled.

In this study, the HDPE, stainless steel, and PVC surface materials were chosen based on their use in commercial tomato packing operations. In a commercial tomato packing operation, different materials are chosen based on different tasks and are not interchangeable. For example, PVC for conveyor belts will not be used in exchange for the HDPE usually used in the bins used to gather tomatoes during harvest. In this study, the types of surface areas used were not compared; however, when the inoculum was transferred from the tomato to the surface, survival on both the tomato and surface showed significant differences ($p < 0.05$). Many studies have been

done to observe pathogen viability and survival on hard surfaces such as these (28, 52, 60, 80, 81, 88, 92). As seen in Table 3.3, the transfer coefficients for 24 h dry treatments were less than 1.0 after 7 d of contact time, except for transfer seen from tomato to PVC (1.6). The nature of the PVC itself could have been a factor. Intrinsically porous materials, such as the PVC used in this study that had the highest survival, allowing bacteria to enter and colonize the material, and enhancing survival. The pores can suction the moisture by capillary action (29). In a study done to observe *Salmonella* colonization on conveyor belts, PVC belt material with the least surface area and fewer pores available for colonization had lower numbers of *Salmonella* remaining than the PVC with greater surface area and more pores (80). *Salmonella* may adhere to both stainless steel and PVC surfaces in such a way that even biocides may not be completely effective (84). The characteristics of HDPE and stainless steel, such as hydrophobicity, should be taken into account in considering how the surface should be maintained and sanitized when used for the transportation of fresh produce. Studies have shown that foodborne pathogens can readily adhere and form biofilms, enhancing survival capability onto hydrophobic food contact surfaces (34, 55, 69, 76).

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CHAPTER 4

CONCLUSION

Surface to surface contact throughout a tomato packing operation can allow for pathogen cross-contamination throughout an entire operation. The results of this study showed that allowing *Salmonella* to dry decreased initial survival on the inoculated surface; however, *Salmonella* was able to survive over time when held between two surfaces. Inoculated surfaces were able to contaminate uninoculated surfaces when the two were contacted. The highest risk for cross-contamination was seen under wet conditions; therefore, maintaining a dry environment may reduce chances for cross-contamination.

Table 3.1. Transfer coefficients for *Salmonella* between white polyvinyl chloride (HDPE) and tomatoes stored at 25°C for up to 7 days. Moisture conditions: inoculated surfaces were wet or dried for either 1 or 24 hours.

Moisture Condition	Tomato to HDPE			
	0 d	1 d	4 d	7 d
Wet	0.78 ^a	1.13 ^a	0.80 ^a	0.83 ^a
1 h dry	1.06 ^b	0.47 ^b	0.41 ^b	0.47 ^b
24 h dry	0.03 ^c	0.10 ^c	1.13 ^c	0.21 ^c

Moisture Condition	HDPE to Tomato			
	0 d	1 d	4 d	7 d
Wet	0.98 ^a	0.96 ^a	0.73 ^a	0.85 ^a
1 h dry	0.86 ^b	0.71 ^b	0.91 ^b	0.77 ^b
24 h dry	0.03 ^c	0.10 ^c	0.12 ^c	0.46 ^c

^{abc}Values with different superscripts were significantly different ($p < 0.05$) by Tukey's multiple comparison method.

Table 3.2. Transfer coefficients for *Salmonella* between stainless steel and tomatoes stored at 25°C for up to 7 days. Moisture conditions: inoculated surfaces were wet or dried for either 1 or 24 hours.

Moisture Condition	Tomato to Stainless Steel			
	0 d	1 d	4 d	7 d
Wet	1.16 ^a	0.92 ^a	0.63 ^a	1.07 ^a
1 h dry	0.86 ^b	1.01 ^b	0.56 ^b	0.63 ^b
24 h dry	0.19 ^c	0.09 ^c	0.30 ^c	0.40 ^c

Moisture Condition	Stainless Steel to Tomato			
	0 d	1 d	4 d	7 d
Wet	0.91 ^a	0.93 ^a	0.87 ^a	0.91 ^a
1 h dry	0.70 ^b	0.63 ^b	0.84 ^b	0.63 ^b
24 h dry	0.50 ^c	0.04 ^c	0.29 ^c	0.50 ^c

^{abc}Values with different superscripts were significantly different ($p < 0.05$) by Tukey's multiple comparison method.

Table 3.3. Transfer coefficients for *Salmonella* between white polyvinyl chloride (PVC) and tomatoes stored at 25°C for up to 7 days. Moisture conditions: inoculated surfaces were wet or dried for either 1 or 24 hours.

Moisture Condition	Tomato to PVC			
	0 d	1 d	4 d	7 d
Wet	0.89 ^a	1.03 ^a	1.06 ^a	1.06 ^a
1 h dry	0.68 ^b	0.99 ^b	1.12 ^b	0.91 ^b
24 h dry	0.30 ^c	0.38 ^c	0.22 ^c	0.16 ^c

Moisture Condition	PVC to Tomato			
	0 d	1 d	4 d	7 d
Wet	0.98 ^a	0.92 ^a	0.80 ^a	0.89 ^a
1 h dry	0.36 ^b	0.98 ^b	0.77 ^b	0.73 ^b
24 h dry	0.04 ^c	0.14 ^c	0.37 ^c	0.58 ^c

^{abc}Values with different superscripts were significantly different ($p < 0.05$) by Tukey's multiple comparison method.

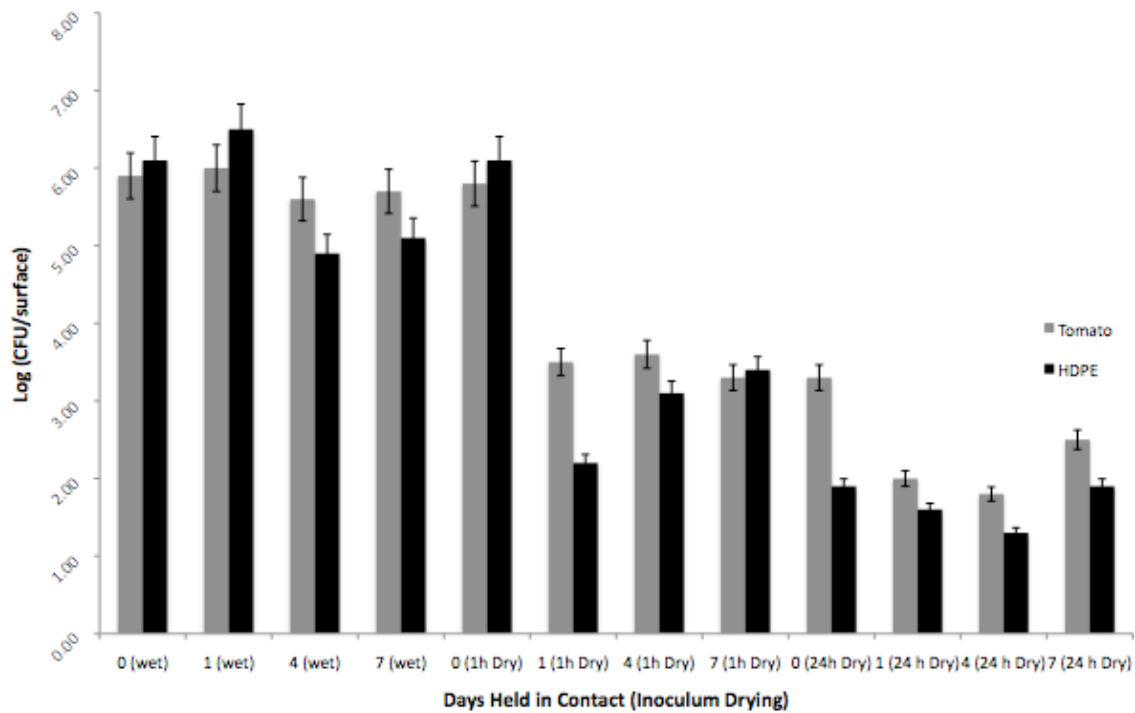


Figure 3.1 Survival of *Salmonella* transferred from inoculated tomatoes to high density polyethylene (HDPE) surfaces under varying conditions when the tomato and HDPE remained in contact for up to 7 days. Wet: inoculated surfaces were wet when contacted to HDPE; 1 h Dry: inoculated surfaces were dried for 1 h before contact to HDPE; 24 h Dry: inoculated surfaces were dried for 24 h before contact to HDPE

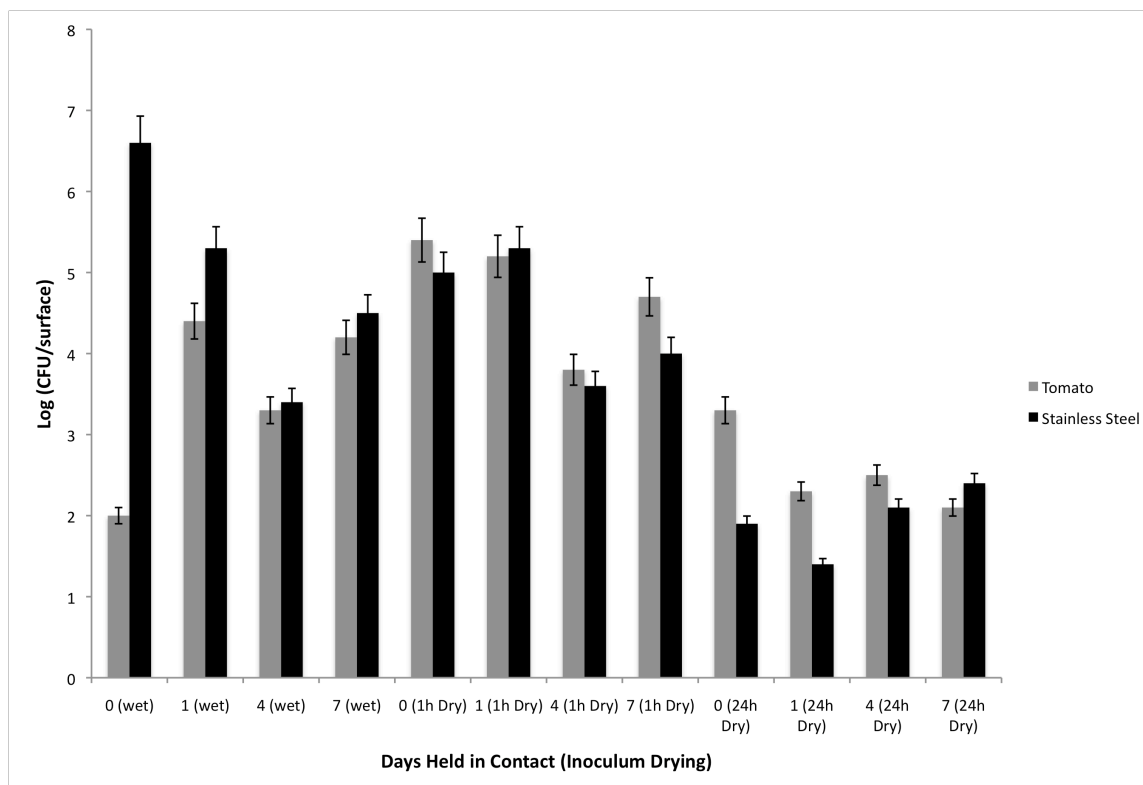


Figure 3.2 Survival of *Salmonella* transferred from inoculated tomatoes to stainless steel surfaces under varying conditions when the tomato and stainless steel remained in contact for up to 7 days. Wet: inoculated surfaces were wet when contacted to stainless steel; 1 h Dry: inoculated surfaces were dried for 1 h before contact to stainless steel; 24 h Dry: inoculated surfaces were dried for 24 h before contact to stainless steel

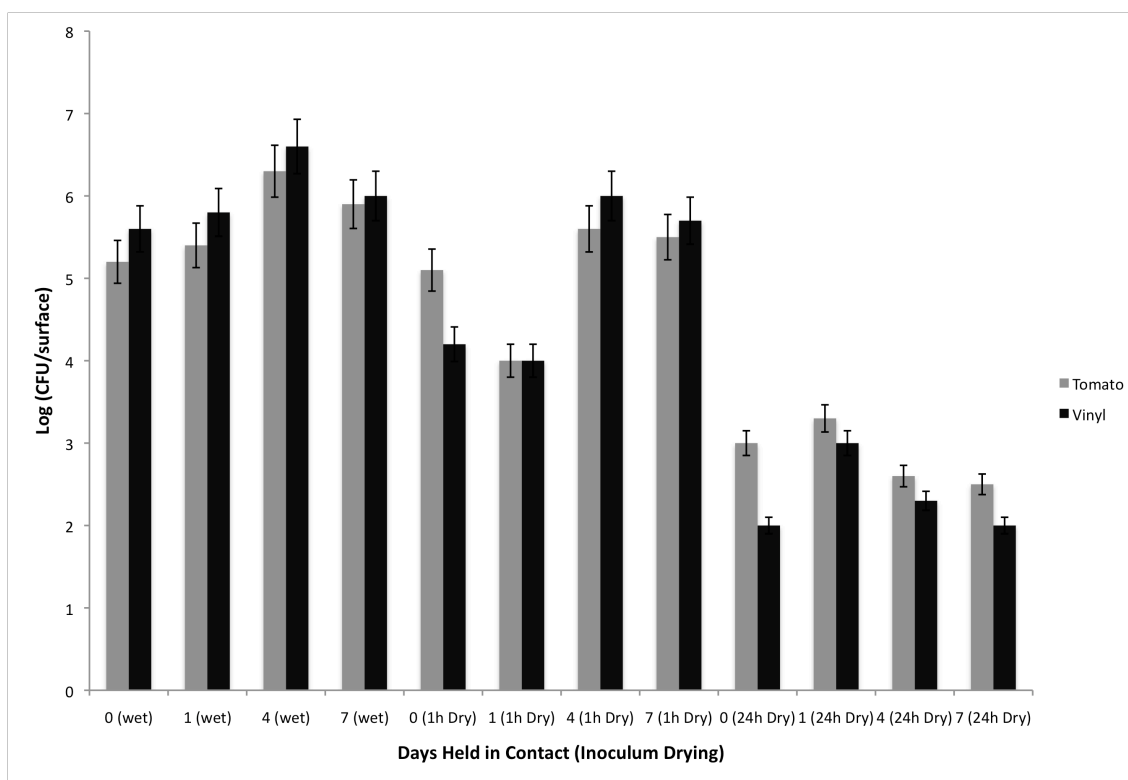


Figure 3.3 Survival of *Salmonella* when transferred from inoculated tomatoes to polyvinyl chloride (PVC) surfaces under varying moisture conditions when the tomato and vinyl remain in contact for up to 7 days. Wet: inoculated surfaces were wet when contacted to PVC; 1 h Dry: inoculated surfaces were dried for 1 h before contact to PVC; 24 h Dry: inoculated surfaces were dried for 24 h before contact to PVC

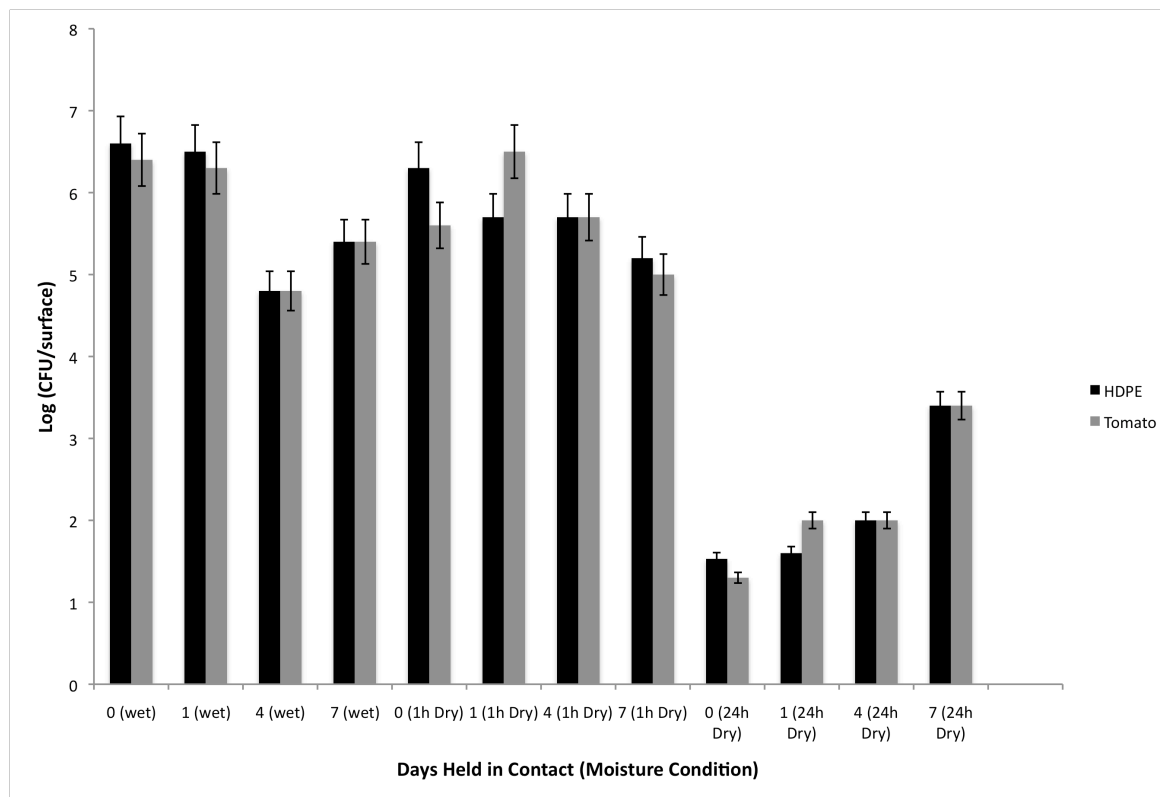


Figure 3.4 Survival of *Salmonella* when transferred from inoculated high density polyethylene (HDPE) surfaces to tomatoes under varying moisture conditions when the tomato and vinyl remain in contact for up to 7 days. Wet: inoculated surfaces were wet when contacted to HDPE; 1 h Dry: inoculated surfaces were dried for 1 h before contact to HDPE; 24 h Dry: inoculated surfaces were dried for 24 h before contact to HDPE

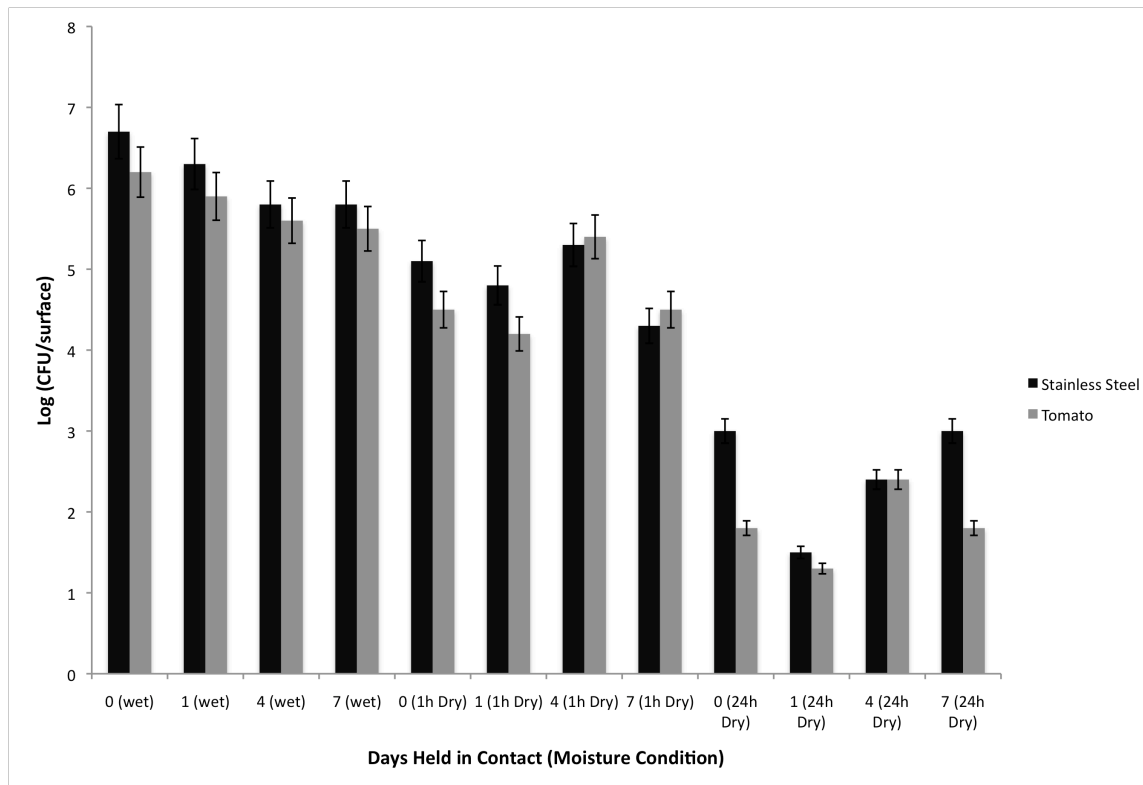


Figure 3.5 Survival of *Salmonella* when transferred from inoculated stainless steel surfaces to tomatoes under varying moisture conditions when the tomato and vinyl remain in contact for up to 7 days. Wet: inoculated surfaces were wet when contacted to stainless steel; 1 h Dry: inoculated surfaces were dried for 1 h before contact to stainless steel; 24 h Dry: inoculated surfaces were dried for 24 h before contact to stainless steel.

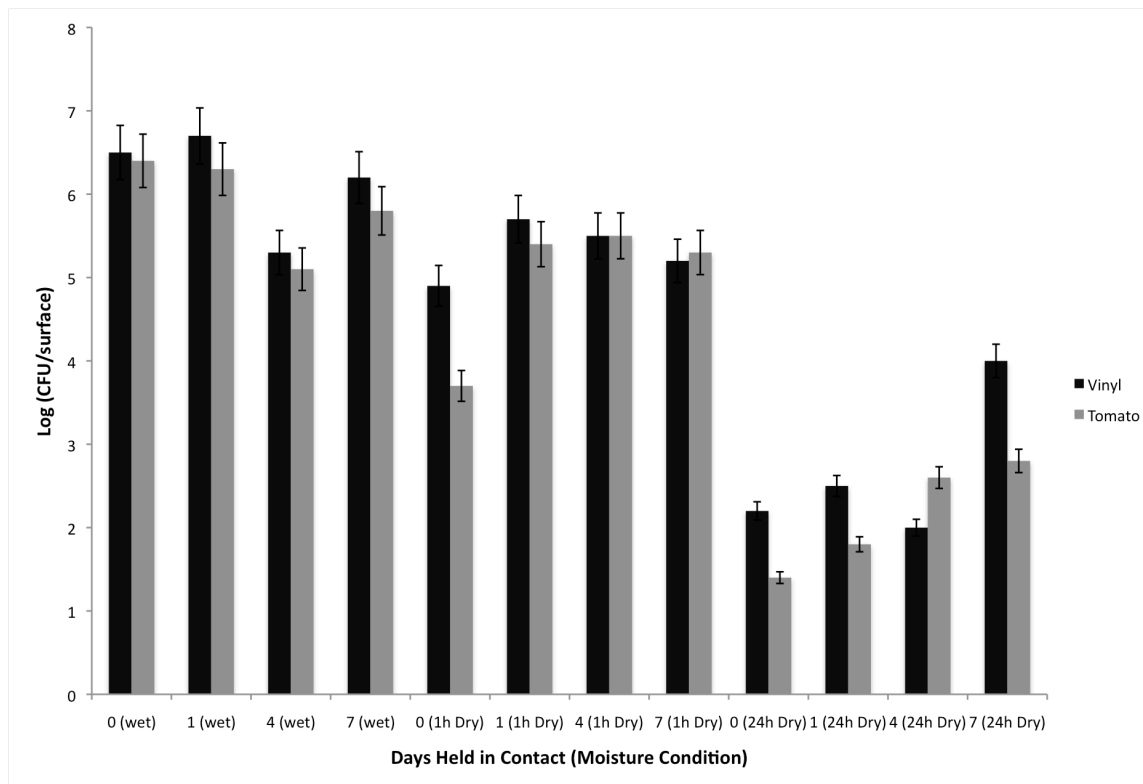


Figure 3.6 Survival of *Salmonella* when transferred from inoculated polyvinyl chloride surfaces to tomatoes under varying moisture conditions when the tomato and vinyl remain in contact for up to 7 days. Wet: inoculated surfaces were wet when contacted to PVC; 1 h Dry: inoculated surfaces were dried for 1 h before contact to PVC; 24 h Dry: inoculated surfaces were dried for 24 h before contact to PVC