Estimating *Salmonella* transfer rates between inoculated ground beef and food contact surfaces with varying contamination levels and serotypes.

by

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Abstract

Salmonella remains a leading cause of foodborne illness associated with beef products. Cross-contamination during food handling represents a critical pathway for transmission. While previous studies have evaluated the individual factors that could influence *Salmonella* transfer, there is limited research examining the combined effects of serotype, inoculation level, and surface type on cross contamination dynamics. Therefore, the objective of this study is to simulate conditions of a typical consumer home kitchen setting to better evaluate how different bacterial levels (high, medium, and low) of distinct *Salmonella* serotypes (Typhimurium, Infantis, Newport, and Dublin) and surface material (hands, wood, plastic, and patty) contribute to cross-contamination. Significant differences ($P<0.0001$) were observed among all three interactions (serotype, treatment, surface). Data presented as percent transfer revealed that transfer efficiency remained consistently high across all surfaces, within treatment, and within serotype. Nearly all observations, with exception to the Mitt sample, approached nearly 100% transfer, indicating minimal loss of microbial level during the processing steps. Although serotype-specific differences were detected, particularly for *Salmonella* Dublin, these did not substantially alter the observed patterns of transfer. Overall, these findings demonstrate that *Salmonella* can be transferred readily between surfaces with high efficiency regardless of initial contamination level, serotype, or surface type. From a food safety perspective, these results underscore the importance of strict hygiene and sanitation practices, as even low levels of contamination may spread and contribute to foodborne illness, particularly in consumer homes.

Keywords: *Salmonella,* percent transfer, cross-contamination, serotype, contamination level, surface type, ground beef.

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Dedication

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Chapter 1 - Literature Review

1.1 *Salmonella enterica*

1.1.1 *Salmonella enterica* subspecies *enterica*

The bacterium *Salmonella* is a flagellated, Gram-negative, rod-shaped, non-spore producing, facultative anaerobe belonging to the *Enterobacteriaceae* family (Han et al., 2024; Jajere, 2019; Oludairo et al., 2022). The *Salmonella* genus is divided into two species, *Salmonella bongori* and *Salmonella enterica* (Han et al., 2024; Jajere, 2019; Oludairo et al., 2022). The *Salmonella bongori* species is primarily associated with cold-blooded animals, particularly reptiles, and are limited to approximately 20 serotypes. In contrast, *Salmonella enterica* has greater diversity and is subdivided into six separate subspecies: *enterica, salamae, arizonae, diarizonae, houtenae, and indica* (Brenner et al., 2000). Among these, *Salmonella. enterica* subspecies *enterica* (henceforth referred to as *Salmonella)* is predominately associated with warm-blooded animals, including livestock and humans, while the other five subspecies are more commonly seen in cold-blooded animals and in the environment (Brenner et al., 2000). *Salmonella* strains are further divided into two categories, Typhoidal and Non-Typhoidal *Salmonella* (NTS). Typhoidal *Salmonella* contains the serovar *Typhi* which is the causative agent of typhoid fever and NTS accounts for the majority of *Salmonella* infections in humans (Jajere, 2019; Moreno Switt et al., n.d.; Oludairo et al., 2022). Salmonellosis, a disease caused by *Salmonella,* is a common foodborne infection that primarily causes self-limiting gastroenteritis, a condition characterized by inflammation of the gastrointestinal tract, causing clinical symptoms such as abdominal cramps and vomiting (Han et al., 2024; Oludairo et al., 2022). To date, there are over 2,500 different serotypes of *Salmonella* that can be determined by their antigenic

properties, and of those 2,500, over 200 serotypes of *Salmonella* have been linked to causing human disease (Oludairo et al., 2022).

1.1.2 *Salmonella* Serotyping

Due to the diversity in the *Salmonella* taxonomy, classification of *Salmonella* strains is an ongoing challenge. Traditional taxonomic approaches define early attempts to classify the pathogen, with a significant advancement in *Salmonella* classification occurring in 1966 when Fritz Kauffmann developed and proposed a serotype-based naming system known as the Kauffman-White Scheme (Oludairo et al., 2022). This scheme allowed for *Salmonella* classification to be based upon categorization of *Salmonella* isolates based on distinct surface antigens that allowed for defining isolates as a "serotype." The Kauffmann-White scheme classifies serotypes based on somatic (O) antigens, flagellar (H) antigens, and capsular (Vi) antigens and is referred to as the antigenic formula (Betancor et al., 2024; Brenner et al., 2000; Glynn et al., 2004; Grimont & Weill, n.d.). One example of how the antigenic formula operates is with the serotype Typhimurium, which has the antigenic formula 1, 4, [5], 12: i: 1,2 where the first number(s) before the first colon represents the O antigen. Subsequently, H antigens are split into two phases, H1 Phase can be found after the first colon and H2 Phase is after the second colon (Grimont & Weill, n.d.). In terms of serotype classification, antigenic characterization provides an important framework for distinguishing *Salmonella* variants. This approach of characterizing *Salmonella* at the serotype level has been successful in classifying *Salmonella* within public heath surveillance and outbreak investigations, even though methods for more specific differentiation between strains have been developed (*i.e.,* whole genome sequencing). Therefore, studies that seek to evaluate the nuances in *Salmonella* ecology largely focus on the observable differences between serotypes.

There are several different serotypes of *Salmonella* that are commonly associated with foodborne diseases. However, Typhimurium and Newport have consistently ranked among the top five most commonly reported serotypes associated with human infection since 2010, with Infantis being ranked in the top ten since 2013 (Collins et al., 2022; Han et al., 2024). These serotypes largely circulate within public health surveillance data due to their close association with food production systems. For example, Typhimurium is one of the most commonly reported serotypes in the food industry (Grimont & Weill, n.d.; Oludairo et al., 2022). This is largely due to its broad host range, including poultry, beef, and pork. Historically, cattle are a major reservoir *Salmonella* (Vanselow et al., 2007). Bosilivac et al. reported that 4.5 % of 173 *Salmonella* isolates recovered from commercial ground beef were identified as Typhimurium (Bosilevac et al., 2009). In the 1990's the CDC declared that Typhimurium accounted for roughly 25% of culture-confirmed infections.

Infantis is one of the five most common serotypes worldwide to affect the food industry. Since 2016, it has been linked to at least 14 outbreaks, with several directly associated with poultry products. While Infantis is most commonly associated with poultry and poultry products, it has also been identified in cattle feedlots, demonstrating its ability to persist across multiple agriculture environments (CDC, 2025; Grimont & Weill, n.d.). Galán-Relaño classified Infantis as the fourth most prevalent serotype with a confirmed 633 cases of human salmonellosis in 2021(Galán-Relaño et al., 2023). The increasing prevalence of this serotype is likely linked to the successful reduction of historically dominant serotypes such as Typhimurium and Enteritidis in the industry (Bassal et al., 2014). As these serotypes decline, ecological niches become available, allowing other serotypes like Infantis to emerge and expand. This is often referred to as serotype replacement. Additionally, Infantis has gained attention due to its association with

antimicrobial resistance and its ability to persist in production environments, further complicating control efforts (Gymoese et al., 2019). This effect with the pathogen is one of the major reasons why completely eradicating *Salmonella* from the food industry is nearly impossible (Bassal et al., 2014).

The formula 6,8,20: e,h: 1,2 represents Newport (Grimont & Weill, n.d.). This serotype is most commonly associated with the consuming uncooked ground beef and has become increasingly common in the industry due to its development of antimicrobial resistance. In addition to causing typical gastroenteritis, antimicrobial resistant strains of Newport present a greater public health concern because infections may be more difficult to treat and can lead to more severe outcomes (Varma et al., 2006). Newport has been associated with the third largest outbreak linked to ground beef (Canning et al., 2023). Between 1996 and 2001 there was an evaluated 7% increase in reported infections and in 2002 roughly 22% of Newport isolates were found to be antimicrobial resistant. (Varma et al., 2006). Beyond its clinical significance, Newport's persistence in food production environments and its potential for transfer between contamination surfaces and food products highlights its importance in cross-contamination studies. Understanding the behavior, survival, and transfer dynamics of this serotype is critical for developing effective intervention strategies aimed at reducing contamination risks within both industrial settings and consumer environments.

Moreover, several serotypes, including Dublin, are not typically classified as a common serotype responsible for human salmonellosis, likely due to its relatively recent emergence. Nevertheless, this serotype has been increasingly associated with more severe clinical outcomes and raises the need for further understanding (Harvey et al., 2017; Velasquez-Munoz et al., 2023) (Grimont & Weill, n.d.). Dublin is considered a host-restricted serotype with a relatively narrow

host range, most commonly associated with dairy cattle with a prevalence of ≥25% (Velasquez-Munoz et al., 2023). This is particularly important given that dairy cows contribute approximately 18% of U.S. ground beef production (Canning et al., 2023). Although less common, infections have also been reported in swine and humans. In human cases, Dublin is associated with severe bacteremia and is notable for having an unusually high mortality rate ranging about 3.9% higher compared to other serotypes (Betancor et al., 2024; Coburn et al., 2007; Harvey et al., 2017; Oludairo et al., 2022; Velasquez-Munoz et al., 2023).

1.1.3 Virulence Factors

Although genetically similar, *Salmonella* serotypes have their differences in pathogenicity due to their variations in virulence factors that influences host colonization, immune evasion, and disease severity. As indicated above, *Salmonella* serotypes differ in host range and in their ability to adhere to host tissues (Betancor et al., 2024). This process is heavily dependent on specialized virulence mechanisms and successful infection of the host, which requires that the strain is able to survive the harsh conditions of the hosts defense system (Han et al., 2024). These mechanisms enhance survival odds, increases colonization and disease development (Han et al., 2024; Jajere, 2019; Moreno Switt et al., n.d.; Velasquez-Munoz et al., 2023).

Salmonella virulence systems are complex and contribute significantly to the severity of infection. A major component of these systems is the presence of *Salmonella* Pathogenicity Islands (SPIs), which are a cluster of genes located on the chromosome of *Salmonella* that encode proteins involved in host cell invasion, intracellular survival and evasion of the host immune response. There are at least 5 major SPIs that have been identified, each contributing to different aspects of *Salmonella* pathogenicity (Coburn et al., 2007; Han et al., 2024; Jajere, 2019;

Velasquez-Munoz et al., 2023). SPI-1is primarily responsible for the invasion of *Salmonella* into the host cells, while SPI-2 plays a role in systemic infections and replication within macrophages. Together, SPI-1 and -2 encode components of the Type III Secretion System (T3SS), which allows *Salmonella* to invade the intestine and spread to systemic sites by injecting bacterial proteins from the *Salmonella* into the host cell (Coburn et al., 2007; Velasquez-Munoz et al., 2023). SPI-3 allows the pathogen to survive within macrophage and in harsh conditions, SPI-4 harbors genes for toxin secretion, and SPI-5 clusters genes that encode T3SS effector proteins (Han et al., 2024; Jajere, 2019). It is important to note that these genes are encoded upon the chromosome, making them highly conserved across *Salmonella* strains and serotypes. This fact is what leads to the assumption that all *Salmonella* serotypes are potentially pathogenic and also contributes to the emergence of *Salmonella* serotypes as new pathogens within the food industry (e.g., *Salmonella* Reading within ground turkey and Dublin within beef products) (Miller et al., 2020).

Beyond the SPIs, other virulence factors are essential for colonization and infection. Adhesion plays a critical role in the early stages of infection by allowing *Salmonella* to attach to tissues (Han et al., 2024; Jajere, 2019). This process is mediated by surface proteins known as adhesins, which bind to receptors on the host cell surface. Adhesion is critical for intestinal colonization and facilitates the function of the T3SS. In addition to adhesins, *Salmonella* produces fimbriae, which are extracellular appendages that are thinner and shorter than common flagella. These structures aid in the pathogens ability to adhere to intestinal epithelial cells and contribute to multiple aspects of pathogenicity (Han et al., 2024; Oliveira et al., 2006).

1.1.4 Economic Impact

NTS remains a significant global public health concern. Similar to typhoid fever, the incidence of intestinal disease caused by NTS is higher in developing countries, yet it continues to pose a substantial burden in developed nations (Coburn et al., 2007). In the United States, NTS is among the top five foodborne pathogens, alongside *Campylobacter, Listeria, Norovirus, and Toxoplasma gondii*, contributing to a considerable proportion of foodborne illness cases. It is estimated to cause approximately 1.35 million illnesses annually and ranks first in terms of economic burden and medical impact (Canning et al., 2023; Hoffmann et al., 2015; Oludairo et al., 2022; Scharff, 2020). More broadly, The Center for Disease Control estimates that 1 in 6 individuals in the United States experiences a foodborne illness each year (Hoffmann et al., 2015).

The economic burden associated with *Salmonella* infections extends beyond healthcare costs to include productivity losses, impacts on the food industry, regulatory expenses, and internation trade distributions. Annual costs attributed to *Salmonella* illness have been estimated to range from $2.3 billion to $11.3 billion, with approximately $294 million associated with hospitalizations alone. Additionally, the public health cost specifically linked to beef products has been estimated at $229 million (Hoffmann et al., 2015; McEntire et al., 2014).

This burden is also evident at the industry level, particularly within the beef sector, where producers must comply with strict regulatory standards while managing the risks associated with contamination and product recalls. Such recalls can lead to substantial financial losses, with estimates reaching up to $178 million depending on the scale of the event. In contrast, the cost of implementing preventive regulatory measures has been estimated at less than $100 million, often making prevention more economically favorable than responding to large-scale recalls (Figure 2)

(Antle, 1998; Shiptsova et al., 2002). The economic impact of foodborne outbreaks has been recognized for decades. For example, Todd et al. evaluated 17 foodborne outbreak-related recalls and found that total direct costs exceeded $575 million, with an individual incidents ranging from $83,000 to more than $164 million (Todd, 1985).

1.2 *Salmonella* in the Beef Industry

1.2.1 Prevalence and Outbreaks in Beef Products

Meat remains one of the most widely consumed food products, with per capita ground beef consumption exceeding 27 pounds annually. In 2019, overall meat consumption increased by approximately 34%, and as consumption continues to rise, so does the potential for *Salmonella* exposure through food products. Notably, in 2021, ground beef accounted for 50% of all beef products sold at retail (Canning et al., 2023). Given its widespread consumption, understanding how *Salmonella* moves throughout the beef production chain is critical for developing effective strategies to prevent foodborne illness. Salmonellosis is a significant disease in cattle, as *Salmonella* is commonly present in the gastrointestinal tracts of food-producing animals and accounts for over 80% of isolates collected from cattle (Ehuwa et al., 2021; Watson et al., 1995). Between 2012 and 2019, beef products were estimated to contribute to approximately 5.75%-9.1% of salmonellosis cases. Non-intact raw beef products, particularly ground beef, were the primary contributors, accounting for 44% of documented illnesses(Canning et al., 2023).

Further evidence highlights the presence of *Salmonella* in retail beef products. Vipham et al. analyzed 2,885 sample collected from 38 cities across the United States and reported *Salmonella* detection in 0.55% of ground beef samples and 1.02% of whole-muscle cuts (Figure (Vipham et al., 2012). Additionally, between 2012 and 2019, a total of 27 *Salmonella* outbreaks

were linked to beef consumption involving 12 different serotypes. Among these, Newport accounted for the highest proportion of outbreaks (26%), followed by Typhimurium (22%). Notably, 12 out of the 27 outbreaks were directly linked to ground beef products, four of the 12 outbreaks caused by Newport, four caused by Typhimurium, and one caused by Dublin (Canning et al., 2023).

1.2.2 Cross Contamination in the Beef Industry

There are multiple points in the beef processing chain that can lead to contamination. To begin, cattle naturally shed *Salmonella* in their feces at levels that can exceed 10^6 CFU/g. The hides of the animals will typically become coated with those feces and are likely to transfer onto the carcasses at slaughter when they are removed (Arthur et al., 2008). More so, in terms of ground products, multiple carcasses are used when producing retail ground beef. If one animal is carrying the *Salmonella* bacteria it can quickly spread across all ground beef products. Which leads to the grinding process of ground beef, allowing contamination to mix through the beef.(Canning et al., 2023). Canning et al. evaluated between 2012 and 2019, 27 *Salmonella* outbreaks were linked to beef consumption which resulted in over a 1000 illness and over 250 hospitalizations (Figure 1)

The goal of studies trying to identify contamination patterns in these facilities is so that the implementation of proper interventions resulting in a significant reduction of pathogens that could potentially enter the food supply (Narváez-Bravo et al., 2013). There are multiple methods of intervention, as simple as wearing the proper PPE during slaughter and insuring proper sanitation. The introduction of HACCP Systems in 1996 was crucial in the implementation of interventions throughout the processing steps to ensure sufficient reductions in the detection of *Salmonella*. The United States Department of Agriculture Agricultural Service (USDA AMS)

also requires facilities to remove the major lymph nodes of cattle prior to grinding process due to its persistence. The USDA has also applied a zero-tolerance rule for *Salmonella* in ground beef products and requires every lot to be tested prior to shipping and distribution (Canning et al., 2023).

1.3 Consumer Handling and Preparation

1.3.1 Cross Contamination in the Consumer Home

While it is the goal to reduce *Salmonella* prevalence in food products before they enter retail, there are still an alarming number of outbreaks that are associated with the cross-contamination of pathogens. There are a multitude of ways that cross contamination can occur, with surface to food by contact (SFC) being reported as a major contributor in causing food-borne illness (Wang et al., 2015).

Salmonellosis is described as being linked to the consumption of food products that have been contaminated with *Salmonella* (Ehuwa et al., 2021). With beef products, ranking fourth to most likely cause salmonellosis, is considered a very important vectors of *Salmonella* infections, due to the likeliness of improper cooking of products that may contain significant levels of the bacteria (McEntire et al., 2014). The most common setting for ground beef preparation with an alarming rate of 67% of outbreaks being in the consumer home (Canning et al., 2023). Cooking beef to an internal temperature of 160 degrees Fahrenheit, handwashing, and avoiding cross-contamination in the consumer kitchen are key interventions that reduce the spread of *Salmonella* in ground beef (Canning et al., 2023). A food safety survey done by the FDA in 2016 showed that 67% of consumers owned a cooking thermometer but only 10% of those consumers actually used the thermometer to check the internal temperature of hamburgers (Canning et al., 2023; Phang & Bruhn, 2011).

The most common way for *Salmonella* contamination is through poor hygiene during handling and preparation and while consumers claim to keep sanitary conditions and surfaces in their homes it was observed that consumers do not properly wash their hands or clean surfaces that have come into contact with raw meat (Phang & Bruhn, 2011). In addition, the surprising number of consumers who have some or have no understanding of cross contamination prevention while handling raw meat products, a study conducted showed that out of the total observed, 76% of participants conducted an action during the process of making hamburgers that could cause cross-contamination (Phang & Bruhn, 2011). *Salmonella* has also shown so have a high transfer rate across different surfaces in the consumer home kitchen with many outbreaks being linked to the *Salmonella* biofilm cells on contact-surfaces to food items (Wang et al., 2015). Cross contamination in the consumer home is not well-researched or quantified for *Salmonella* in ground beef products (Strickland et al., 2023). It is crucial to understand these processes in attempts to further reduce salmonellosis incidences for consumers.

Due to these factors, the objective of this study was to simulate a consumer home kitchen environment and evaluate how different *Salmonella* serotypes, at varying inoculation levels and across multiple surface types, interact to influence cross-contamination and bacterial transfer. While previous research has largely examined these variables independently, limited information exists on how they function in combination under realistic conditions. Therefore, this study aims to address this gap by providing a more comprehensive understanding of the complex interaction that drives *Salmonella* transmission in domestic settings. The findings from this work are intended to inform risk assessment models and support the development of more effective intervention strategies to reduce foodborne illness at the consumer level.

1.4 Figures and tables

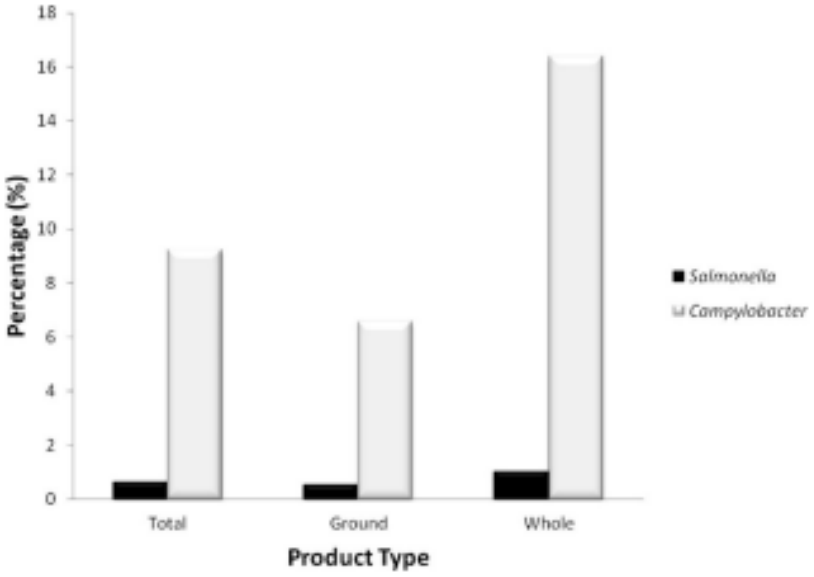

Figure 1.1. Baseline values of retail samples testing positive for *Salmonella* and *Campylobacter* for all samples (n = 4,070) and by product type (ground beef, n = 3,152; whole muscle, n = 918). All samples were tested for the presence of *Salmonella* (n = 2,885). A subset (n = 1,185) was tested for *Campylobacter* by Vipham et al. (2012)

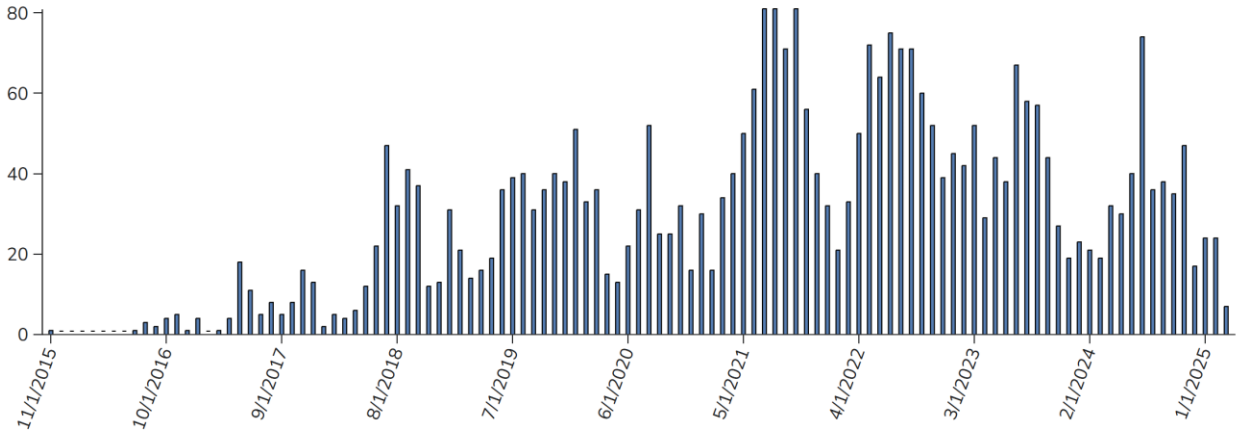

Figure 1.2 People with *Salmonella* Newport illnesses caused by the REPJJP01 strain, by date illness began, 2015-2025 by CDC (2025)

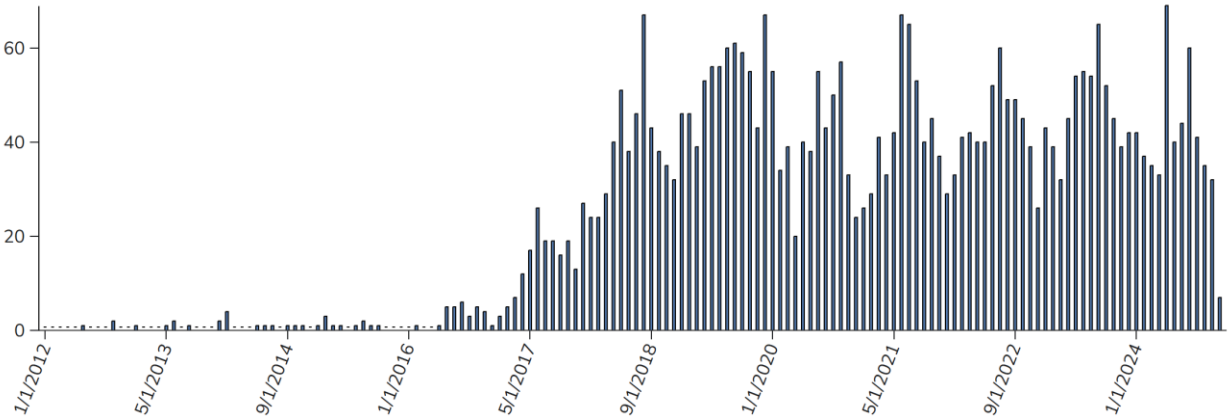

Figure 1.3 People with *Salmonella* Infantis illnesses caused by the REPJFX01 strain, by month of isolate collection, 2012-2024 by CDC (2025)

Table 1.1. Characteristics of *Salmonella* outbreaks associated with beef, by beef type , Foodborne Disease Outbreak Surveillance System, United States, 2012-2019 by Canning et al. (2023)

Characteristic	*N*	Ground, *n*	Intact Raw, *n*	RTE Beef, *n*	Other Types[A] *n*	Unspecified, *n*
Epidemiology						
Outbreaks	27	12 (44%)	6 (22%)	2 (7%)	4 (15%)	3 (11%)
Illnesses	1103	800 (73%)	26 (2%)	24 (2%)	74 (7%)	179 (16%)
Median per outbreak (range)	13 (2–436)	32 (3–436)	4 (2–7)	12 (6–18)	17 (8–32)	38 (4–137)
Hospitalizations available[B]	834 (76%)	598 (75%)	26 (100)	23 (96%)	69 (93%)	118 (66%)
Hospitalized	254 (30%)	221 (37%)	10 (38%)	1 (4%)	9 (13%)	13 (11%)
Deaths	2	2 (100%)	0	0	0	0
Patient sex available[B]	927 (84%)	790 (99%)	26 (100%)	24 (100%)	45 (61%)	42 (23%)
Female	466 (50%)	397 (50%)	12 (46%)	22 (92%)	15 (33%)	20 (48%)
Patient age available[B]	911 (83%)	762 (95%)	26 (100%)	22 (92%)	61 (82%)	40 22%)
<1 year	17 (2%)	17 (2%)	0	0	0	0
1 to 4 years	44 (5%)	40 (5%)	0	0	2 (3%)	2 (5%)
5 to 9 years	38 (4%)	30 (4%)	0	1 (5%)	2 (3%)	5 (13%)
10 to 19 years	105 (12%)	88 (12%)	3 (12%)	1 (5%)	10 (16%)	3 (8%)
20 to 49 years	360 (40%)	278 (36%)	19 (73%)	11 (50%)	28 (46%)	24 (60%)
50 to 74 years	286 (31%)	253 (33%)	2 (8%)	7 (32%)	18 (30%)	6 (15%)
75 years and up	61 (7%)	56 (7%)	2 (8%)	2 (9%)	1 (2%)	0
Outbreak Investigation						
Scope						
Single state	19 (70%)	4 (33%)	6 (100%)	2 (100%)	4 (100%)	3 (100%)
Multistate	8 (30%)	8 (67%)	0	0	0	0
Duration						
Median outbreak duration (days), range	12 (1–288)	63 (2–288)	8 (1–79)	5 (3–7)	10 (2–15)	14 (6–86)
Median investigation duration[C] (days), range	95 (60-217)	95 (60-217)				
Setting of food preparation[D]						
Private home	12	8	1			3
Restaurant	8	1	4	1	2	
Caterer	1				1	
Religious Facility	1				1	
Grocery store	1		1			
Other	1			1		
Unknown	1	1				
Multiple	2	2				

Table 1.2. Annual Losses for the Poultry, Beef, and Pork Industries by Shiptsova et al. (2002).

Industry	Year	Actual Losses		Actual and Expected Losses	
		Lower limit	Upper limit	Lower limit	Upper limit
Pounds					
Pork	1996	41,765	200,615	45,042	203,892
	1997	33,000	1,351,026	273,342	1,591,368
	1998	491,512	39,119,626	1,234,880	39,862,994
	1999	140,587	33,887,056	302,999	34,049,468
Beef	1996	168,374	327,224	171,651	330,501
	1997	25,709,958	27,027,984	25,950,300	27,268,326
	1998	2,050,410	40,678,524	2,793,778	41,421,892
	1999	1,241,602	34,988,071	1,404,014	35,150,483
Poultry	1996	190,320	349,170	193,597	352,447
	1997	474,702	1,792,728	715,044	2,033,070
	1998	305,892	38,934,006	1,049,260	39,677,374
	1999	713,242	34,459,711	875,654	34,622,123
Millions of $					
Pork	1996	0.293	1.407	0.316	1.429
	1997	0.081	9.930	2.009	11.697
	1998	1.193	284.830	8.991	290.242
	1999	0.340	245.512	2.195	246.688
Beef	1996	0.472	2.751	1.443	2.778
	1997	215.578	226.630	217.593	228.645
	1998	5.682	338.161	23.225	344.340
	1999	3.573	302.087	12.122	303.489
Poultry	1996	0.814	1.493	0.828	1.507
	1997	2.036	7.690	3.067	8.721
	1998	1.332	169.536	4.569	172.773
	1999	3.128	151.128	3.840	151.840

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Chapter 2 - Estimating *Salmonella* Transfer Rates Between Inoculated Ground Beef and Food Contact Surfaces with Varying Contamination Levels and Serotypes.

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2.1 Introduction

Non-typhoidal *Salmonella* is one of the most significant pathogens associated with foodborne illness in the world, responsible for an estimated 1.3 million infections in the United States alone every year (Centers for Disease Control and Prevention, 2024; Bhunia, 2018). Salmonellosis, the human disease caused by *Salmonella*, is a common foodborne infection that causes gastroenteritis symptoms such as diarrhea, fever, and abdominal cramps within 12-72 hours following exposure (US. Food and Drug Administration n.d.). While most healthy individuals tend to recover from salmonellosis without the need for extensive treatments, the illness can be detrimental and life threatening for vulnerable populations including young children, the elderly, and immunocompromised individuals (U.S. Food and Drug Administration n.d.). In serious cases, a *Salmonella* infection may progress beyond the gastrointestinal tract and can cause serious conditions such as bacteremia which is the presence of bacteria in the bloodstream (Betancor et al., 2024; Coburn et al., 2007; Glynn et al., 2004; Velasquez-Munoz et al., 2023). The World Health Organization (WHO) reports that 1 in 10 people fall ill due to foodborne disease and 420,000 die annually. Of the foodborne diseases, *Salmonella* is estimated to be 1 of the 4 main causes of diarrheal diseases and in 2019 was estimated to cause 79,000 deaths annually (WHO, 2018, 2022, 2024). While these estimates demonstrate the clinical significance and overall pathogenic potential of *Salmonella,* understanding the ecology of *Salmonella* within the food industry and public health requires a deeper understanding of its taxonomy and serotype diversity.

The *Salmonella* taxonomy is complex with two species (*Salmonella bongori* and *Salmonella enterica*) and six sub-species (Tindall et al., 2005). The primary concern for the food industry is Non-typhoidal *Salmonella enterica subsp. enterica* (henceforth referred to as

Salmonella), which alone contains over 2,500 serotypes (Switt et al., 2009). Among these serotypes, there are a subset that are most commonly associated with human cases of salmonellosis. In 2022, CDC surveillance data collected from food related outbreaks indicated that of the 5,442 *Salmonella* isolates that were serotyped, the most common serotypes were Newport (11%) and Typhimurium (9%) followed by Infantis (4%) (Collins et al., 2022). In 2019 Typhimurium had 6,287 laboratory confirmed samples (Scallan Walter et al., 2025). The CDC reported that between the years of 2015-2025 Newport had documented 3,325 illnesses and 14 Outbreaks (Figure 2.1). Infantis had 3,904 reported illnesses and 14 outbreaks between 2012-2024 (Figure 2.2) (CDC, 2025a, 2025b). Moreover, while *Salmonella* Dublin is not recognized as a common serotype to cause human salmonellosis due to its relative novelty, an analysis conducted by Harvey et al. between 1996-2013 showed hospitalizations due to this serotype had increased from 68% to 78% (Harvey et al., 2017). While there is variation in the prevalence of *Salmonella* serotypes, there is also considerable diversity in their biological behavior.

This variability is largely driven by the differences in pathogenicity. The pathogenicity (i.e., ability to cause disease) of *Salmonella* is largely due to its virulent factors often found on *Salmonella* Pathogenicity Islands (SPIs), which are clusters of genes on the chromosome that aid in the invasion, replication, survival and evasion within host cells (Jajere, 2019). *Salmonella* pathogenicity is highly serotype dependent and enhances the pathogens' adaptability and persistence within food systems. This variation also complicates *Salmonella* detection, control, and overall prevention in the food industry (Han et al., 2024; Moreno Switt et al., n.d.; Velasquez-Munoz et al., 2023) Much of this variability is driven by differences among serotypes, which exhibit distinct survival and transmission characteristics. *Salmonella* can be transmitted through multiple routes, most commonly through the consumption of contaminated food

products. Environmental exposure through contaminated surfaces or food preparation equipment also tends to contribute to the exposure and spread of this pathogen (Antle, 1998; Kusumaningrum et al., 2004; Phang & Bruhn, 2011; Wang et al., 2015).

On average the *Salmonella* prevalence in beef is roughly 2.4%, as demonstrated by a study conducted between 2005-2007 (Bosilevac et al., 2009). Strickland et al. further shows that an estimated total of 8980 illnesses a year can be linked to ground beef consumption. This same study also revealed that around 6,259 illnesses were caused by ground beef products consumed in the consumer home (Strickland et al., 2023). Bosilevac et al. also demonstrated that of the 173 of serotypes collected from commercial ground beef, common serotypes linked to human foodborne disease were observed with Typhimurium (4.5%) being the highest of this subset, next directly after was Dublin (3.4%), then Infantis (1.7%), which was followed by Newport (1.7%) were the commonly seen (Bosilevac et al., 2009).

One of the primary causes of salmonellosis and foodborne disease is cross-contamination, which allows pathogens to spread from contaminated sources onto clean foods and surfaces (Kusumaningrum et al., 2004). However, despite the existence of consumer guidelines designed to mitigate this risk, compliance in household settings is often inconsistent, thereby sustaining the potential for pathogen transmission (Phang & Bruhn, 2011). For example, in a study conducted by Phang et al. which evaluated videotapes from the household of 199 volunteers over the age of 18 and of varying demographics and education levels, had determined that less than half of observed consumers demonstrated adequate understanding of proper food preparation and safe cook times, and less than 10% of consumers met the recommended hand-washing guidelines (Phang & Bruhn, 2011; Strickland et al., 2023). Additional research on serotype adhesion to surface types has shown that varying serotypes behave differently and adhesion levels on

selected contact surfaces are strain dependent and influences contamination. This study by Oliveira et al. evaluated a significant difference in the adhesion density of four different strains of *Salmonella* Enteritidis and three select surface materials (Oliveira et al., 2006). This highlights the complexity of *Salmonella* and emphasizes the importance of targeted interventions, such as safe handling practices and proper sanitation protocols, to reduce contamination risks in both the food industry and consumer homes. Effectively developing these interventions requires a clearer understanding of how multiple factors collectively influence *Salmonella* cross-contamination.

While studies have been conducted to examine these factors individually, there remains a gap in the research regarding how varying bacterial levels, *Salmonella* serotypes, and surface materials in combination would impact cross-contamination outcomes (Iulietto & Evers, 2020). It is important to understand these variables together, as real consumer kitchens contain multiple interacting factors occurring simultaneously rather than in isolation. Evaluating their combined effects can provide a more realistic assessment of cross-contamination risks and better inform food safety interventions and consumer guidance. Therefore, the objective of this study is to simulate conditions of a typical consumer home kitchen setting to better evaluate how different bacterial level (high, medium, and low) of distinct *Salmonella* serotypes (Typhimurium, Infantis, Newport, and Dublin) and surface material (stainless steel, hands, wood, and plastic) as well as the contaminated meat itself, contribute to cross-contamination. By assessing these factors collectively, this study aims to provide an understanding of contamination risks present in the everyday consumer's home kitchen. Findings in this research will also support public health initiatives and consumer education with the goal of reducing the incidence of salmonellosis

2.2 Materials and Methods

2.2.1 Cell Growth

For this study, a standardized cell growth protocol from the Schmidt Lab at USMARC was utilized (Schmidt 2025). *Salmonella* cells were cultured to produce the inoculum that was used for mixing into ground beef. Each week, one of four serotypes of *Salmonella enterica* subsp. *enterica* (Typhimurium, Infantis, Newport, and Dublin) were selected and cultured to create three different inoculum levels, 6 Log, 5 Log, and 4 Log (Henceforth referred to as High, Medium, and Low respectively). For inoculum preparation, serotypes were streaked in duplicate onto Brain Heart Infusion (BHI) Agar plates (Becton, Dickinson and Company, Franklin Lakes, NJ). Plates were then incubated at 35°C for 18-24 hours.

Following incubation, BHI plates were removed from the incubator, and a single isolated colony was selected from each plate and placed into 9 mL of BHI broth (Becton, Dickinson and Company, Franklin Lakes, NJ). *Salmonella* culture tubes were placed back into the 35°C incubator for an additional 22 hours. After incubation, tubes were removed, and optical density (OD) was measured using a spectrophotometer (Thermo Fisher Scientific, Waltham, MA) to ensure cultures reached an OD of at least 0.4 at 600 nm (dilution factor 1.0). Once the target OD was achieved, 220 µL from each culture was transferred into 22 mL of Buffered Peptone Water (BPW) (Romers Labs, Union, MO) and incubated for 2.5 hours at 35°C. After incubation, OD was measured again with an expected OD of at least 0.05 at 600 nm (dilution factor 1.0). If samples fell below the 0.05 target, they were returned to the 35°C incubator in 15-minute intervals. OD was reassessed after each interval until the desired value was obtained.

2.2.2 Creating Inoculum

From the culture tubes, the tube that met the target OD value was selected and centrifuged at 3000 x g for 10 minutes. Following centrifugation, the supernatant was carefully poured from the tube without disrupting the pelleted bacterial bead. The resulting *Salmonella* pellet was resuspended in 20 mL of Phosphate-Buffered Saline (PBS) (VWR, Radnor, PA) and labeled "Mother Tube". From the Mother Tube serial dilutions were created by transferring 4 mL from the Mother Tube into 36 mL of PBS. Inoculums 10^6, 10^5, and 10^4 (dilutions -2, -3, and -4 respectively) were used for inoculating the ground beef samples. These levels were selected based on prior pilot studies, which determined that they produced optimal countable ranges. Additionally, inoculums 10^2 and 10^1 (dilutions -6 and -7 respectively) were plated onto Aerobic Plate Count (APC) Petrifilms® (Neogen, Lansing, MI) for concentration verification. Per AOAC 2015.13, APC petrifilms were incubated at 35°C for 24 ± 2 hours to verify initial bacterial level at 10^8 CFU/mL.

2.2.3 Processing

Each week, 30 one-pound packages of ground beef (N=30) were inoculated using the selected inoculum levels (High, Medium, and Low).

Prior to inoculation, 25 grams were aseptically collected from each individual one-pound package (~453 grams) of ground beef to serve as the Negative Control sample. Each sample was placed into a sterile, filtered Whirl-Pak® bag (Fisher Scientific, Waltham, MA**)** and enriched with 225 mL of BPW. Negative Control samples were then incubated at 42°C for 24 ± 2 hours. The remaining ground beef (~428 grams) was transferred into a stainless-steel bowl and inoculated with one of the three *Salmonella* levels (High, Medium, or Low). The ground beef was mixed using a Kitchen Aid mixer for two minutes to ensure distribution of the inoculum and

then allowed to rest for 30 minutes to establish optimal microbial attachment. After resting, a 25-gram portion was removed to serve as Positive Control and 225 mL of BPW was added for enrichment.

To mimic hand transfer, Micro Tally Mitts® (Fremonta, San Jose, CA) (Sample 1) were used to collect a 100-gram portion of the inoculated ground beef. These 100-gram portions were rolled into balls and flattened into patties onto cutting board surfaces using a patty template. Two patties were placed on a wood cutting board (Sample 2) and two patties were placed on a plastic cutting board (Sample 3). A 10 cm x 10 cm area was sampled on both the wood and plastic cutting boards using a BPW-enriched sponge (Neogen, Lansing, MI). Mitts were placed into sterile bags and enriched with 90 mL of BPW.

Portions of each patty from both cutting boards were collectively gathered to obtain a 25-gram sample. This composite was designated as the Patty sample (Sample 4) and enriched with 225 mL of BPW. After the patties were removed from the cutting boards, a 10 cm x 10 cm area from each cutting board surface was sampled using a BPW-enriched sponge.

All samples (1-4 as well as positive and negative controls) were homogenized for 60 seconds and then incubated at 42°C for 24 ± 2 hours. Following enrichment and homogenizing, High-level surface-type samples were used to prepare serial dilutions, and the 10^0 and 10^{-1} dilutions were plated in duplicate. For Medium- and Low-level samples, the 10^0 dilution was plated in quadruplicate onto XLD agar (Remel, Lenexa, KS) using a spread plating technique. All XLD agar plates were incubated at 35°C for 18-24 hours.

2.2.4 Data Collection

After incubation, surface-type samples were removed from the incubator and processed to generate detection lysates. Lysis reagent was prepared by adding 150µL of reagent to 12 mL

of lysis buffer (Hygiena, Camarillo, CA) and distributed in 200 µL increments into cluster tubes. Subsequently, 5 µL of the enrichment was added to the 200 µL of lysis reagent and placed onto a thermocycler to complete 20 min at 37 °C, 10 min at 95 °C, and cooling to 4 °C for 5 min. Following lysis, samples were analyzed using The BAX® real-time PCR system (Hygiena, Camarillo, CA) to confirm the absence of *Salmonella* in the Negative Control samples and for the presence of *Salmonella* in Positive Control samples and in remaining surface-type samples.

XLD agar plates were removed, and isolated black *Salmonella* colonies were counted to obtain quantitative data. Results were reported in logarithmic form (log CFU/unit). Log CFU/g values were calculated for weighed samples (positive control and patty) and the Mitt sample, Log CFU/cm^2 was calculated for sponge samples (wood, and plastic).

$$\text{Log CFU/g} = \log\left(avg * \frac{10^{(dilution)}}{volume\ plated}\right) * \left(\frac{rinse\ volume}{weight\ of\ sample}\right)$$

$$\text{Log CFU/cm}^2 = \log\left(avg * \frac{10^{(dilution)}}{volume\ plated}\right) * (100)$$

2.2.5 Experimental design and statistical analysis

This study utilized a 3-way Factorial design, with three inoculation levels, within four serotypes, across four surface types. Batches were considered the experimental unit, with treatments defined by *Salmonella* serotype (Typhimurium, Infantis, Dublin, Newport) and inoculation level (High, Medium, Low). The dependent variable was colony counts from XLD plates. A total of 117 samples were analyzed for this study. Three batches were omitted due to Negative Controls testing positive prior to inoculation.

A generalized linear mixed model (GLIMMIX) was utilized to evaluate differences in Log CFU count data and transfer rate calculations. Fixed effects included *Salmonella* level, serotype, and surface type, along with the corresponding two-way and three-way interactions. A

random effect of batch nested within treatment was included to account for variation among experimental batches. The model assumed Gaussian distributions and model parameters were estimated using restricted maximum likelihood. Degrees of freedom were calculated using the Kenward-Roger method. Statistical significance was determined by an $\alpha=0.05$. All data were analyzed using Statistical Analysis Software version 9.4 (SAS Institute, Cary, NC, USA).

2.3 Results and Discussion

This study evaluated the transfer of four *Salmonella* serotypes (Typhimurium, Infantis, Dublin, Newport), three different inoculation levels (high, medium, low), across four surfaces (Mitt, Plastic, Wood, Patty) to better understand how these variables interacted to influence cross-contamination. This approach was designed to simulate realistic food handling conditions in a consumer home kitchen and to address gaps in the literature, where serotype, contamination level, and surface type are often studied independently rather than in combination.

Figure 2.1 illustrates the APC counts for each of the initial inoculums used to create treatment levels high, medium, and low for each serotype. Serotype Typhimurium had the highest values (8.31 Log CFU/mL), followed by Newport (8.21 Log CFU/mL), next was Dublin (7.97 Log CFU/mL), and ending with Infantis with the lowest initial inoculum microbial level (7.87 Log CFU/mL). Although each serotype was subjected to the same cell growth protocol these differences in the initial microbial level among serotype inoculums are important to consider, as they directly influence the resulting Log CFU/Unit values observed across the high, medium, and low treatment levels within each serotype.

Salmonella inoculation levels in ground beef are presented in Figure 2.2. Results demonstrated a significant two-way interaction between serotype and treatment ($P<0.0001$), indicating that the effect of treatment on *Salmonella* recovery from Positive Controls differed

across serotypes. Overall, higher inoculation levels resulted in greater Log CFU/Unit counts. A consistent stepwise pattern was observed for Typhimurium, Infantis, and Newport, where each treatment differed from the others. High levels (4.895, 4.685, and 5.184 Log CFU/g), were significantly greater than medium levels (3.906, 4.183, and 4.048 Log CFU/g), and both were significantly higher than low levels (3.434, 2.761, and 3.677 Log CFU/g). In contrast, Dublin exhibited a slightly different response, as the high and medium levels (4.488 and 4.234 Log CFU/g) were not significantly different from each other, and both levels were significantly greater than the low level (3.157 Log CFU/g). These findings confirm that inoculation levels differed across serotypes and suggest that these differences may contribute to the observed influence of serotype on subsequent surface contamination levels.

Figure 2.3 represents the Bowl samples. Similar to Positive Control, Bowl samples demonstrated a significant two-way interaction between serotype and treatment ($P<0.0001$). For Typhimurium, Infantis, and Dublin, a stepwise relationship was observed, with high levels (5.178, 5.182, and 5.490 Log CFU/mL) being significantly different from the medium levels (4.087, 4.538, 5.110 Log CFU/mL) and both being significantly different from the low levels (3.566, 3.158, and 3.714 Log CFU/mL). Newport also had a significant difference between high (5.074 Log CFU/mL), medium and low levels but was not significantly different between medium and low levels (4.33 and 4.078 Log CFU/mL). These measurements were used to better characterize the influence of the mixing process on inoculum distribution prior to surface transfer.

In terms of *Salmonella* transfer on surfaces, a significant three-way interaction among serotype, treatment, and surface was observed ($P<0.0001$). Figure 2.4 shows the estimated *Salmonella* levels (Log CFU/Unit) for all four *Salmonella* serotypes collectively. Across

treatments, consistent patterns emerged in which the miss surface was significantly different from plastic, wood, and patty samples. For **panel a** high and low treatments, mitt consistently yielded lower recovery, while plastic and wood were often similar but differed from patty, particularly under high and low treatments. In the medium treatment, slight variations were observed, with mitts differing from plastic, and both were significantly different from wood and patty.

Panel b demonstrated that high and medium treatments followed similar trends, with mitt samples significantly different from all other surfaces, while no significant differences were observed among surface types under low treatment. **Panels c** and **d** were identical in that all three treatments shared similarities in differences with mitts being significantly different from plastic, wood, and patty. However, wood and plastic were significantly different from the patty sample.

Lastly, discrepancies between bowl and the other four surface types observed during initial analysis prompted recalculation of the quantifiable data as percentage transfer. **Figure 2.5** presents this data in a format consistent with **Figure 2.4** and demonstrates a similar significant three-way interaction among serotype, treatment, and surface ($P<0.0001$). Recovery of *Salmonella* across all serotypes was consistently high across surface types, with values approaching complete transfer. Regardless of treatment, nearly all observations approached ~100% transfer efficiency. This indicates that there was minimal loss of cells during the transfer between surfaces. Despite the overall high transfer efficiency, there were still some minor differences in the microbial recovery among surface types that were observed. Across all four serotypes, recovery was lowest for the mitt sample, whereas plastic, wood, and patty exhibited similar high transfer rates, with patty often yielding the greatest recovery. Lack of separation

between surface type samples indicates that the transfer efficiency was not strongly influenced by the initial treatment.

This study evaluated the transfer dynamics of four *Salmonella* serotypes across multiple surface types and inoculation levels to better understand factors influencing cross-contamination. Although there were statistically significant differences between serotype, treatment, and surface throughout the study, the most notable finding was the consistently high transfer rate across these conditions. These findings suggest that cross-contamination risk remains substantial even at lower contamination levels (Ehuwa et al., 2021; Kusumaningrum et al., 2004; Phang & Bruhn, 2011). While higher inoculation levels resulted in greater recovery, the percentage transfer data shows that the proportion of cells transferred remains mainly unchanged. This highlights the importance of understanding cross-contamination because even at low levels of contamination, those lower levels may still be sufficient enough to result in an effective transfer and potential downstream exposure.

Surface type may have had an influence on microbial recovery (Oliveira et al., 2006). Mitt surfaces consistently resulted in lower recovery compared to Plastic, Wood, and Pattie surfaces. This reduction may be due to the porous nature of the mitts material, which may cause bacteria to become trapped in the material, and not allowing for a full evaluation of transfer during sampling. In contrast, smoother surfaces of the cutting boards and the overall Patty may have supported a more efficient transfer of cells. Despite these differences, transfer efficiency still remained high and reinforced the idea that surface type alone is affecting the transmission of *Salmonella*.

Differences observed among serotypes, particularly the behavior exhibited by Dublin and Infantis, may be partially explained by the variation observed with the initial inoculum in Figure

1 as well as inherent biological characteristics. Even though subjected to the same protocol and measures, each serotype behaved in a certain way throughout this study. Infantis and Dublin consistently throughout this study demonstrated slightly different responses compared to Typhimurium and Newport which remained fairly constant. This could be due to the attachment, survival, and biological differences of these serotypes as demonstrated by Díez-García et al. which evaluated varying growth curves of common *Salmonella* serotypes often found within the food industry (Díez-García et al., 2012).

It is important to note that although all four serotypes initially reached that 10^8 Log CFU/Unit measurement, each serotype responded differently to the cell culture protocol. This can clearly be seen as even though each serotype was subjected to the same protocol, Infantis and Dublin had a slower growth response compared to Typhimurium and Newport. This is further confirmed in other literature showing that there is a slight difference in growth rates depending on serotype (Díez-García et al., 2012). To compare the effects of both serotypes and surface type, all sampling data were combined. After conducting statistical analysis, a significant three-way interaction was observed between serotype, treatment, and surface.

2.4 Conclusion

From a food safety perspective, these findings underscore the critical importance of strict hygiene and sanitation practices during food handling, particularly in consumer home environments. The consistently high transfer efficiency observed across all serotypes, surfaces, and inoculation levels indicates that even minimal contamination events can readily result in widespread cross-contamination scenarios but remains significant even at low microbial levels.

Importantly, while differences in surface type and serotype behavior were observed, these factors did not substantially reduce the overall efficiency of bacterial transfer. This highlights the

key public health concern that once *Salmonella* is introduced into the food preparation environment, it can be easily spread across multiple contact surfaces regardless of material type or initial contamination level. As a result, reliance on surface characteristics alone as a mitigation strategy is insufficient. These findings further reinforce the need for preventive approaches that focus on reducing initial contamination and interrupting transfer pathways. Proper hand hygiene, effective cleaning and sanitation of food-contact surfaces, and separation of raw and ready-to-eat food remain essential interventions. Additionally, consumer education on safe food handling practices is critical, as improper handling in the home continues to be a major contributor to foodborne illness. From an industry and regulatory perspective, this study further supports the importance of upstream control measures aimed at minimizing *Salmonella* contamination prior to retail. Reducing pathogen level earlier in the production chain can significantly reduce the likelihood of cross-contamination during food preparation.

Overall, this study demonstrates that *Salmonella* transfer is highly efficient under a wide range of conditions, emphasizing that even small lapses in food safety practices can have a meaningful consequence. Therefore, comprehensive and consistent control strategies are essential to reduce the risk of foodborne illness and improve public health outcomes.

2.5 Figures and tables

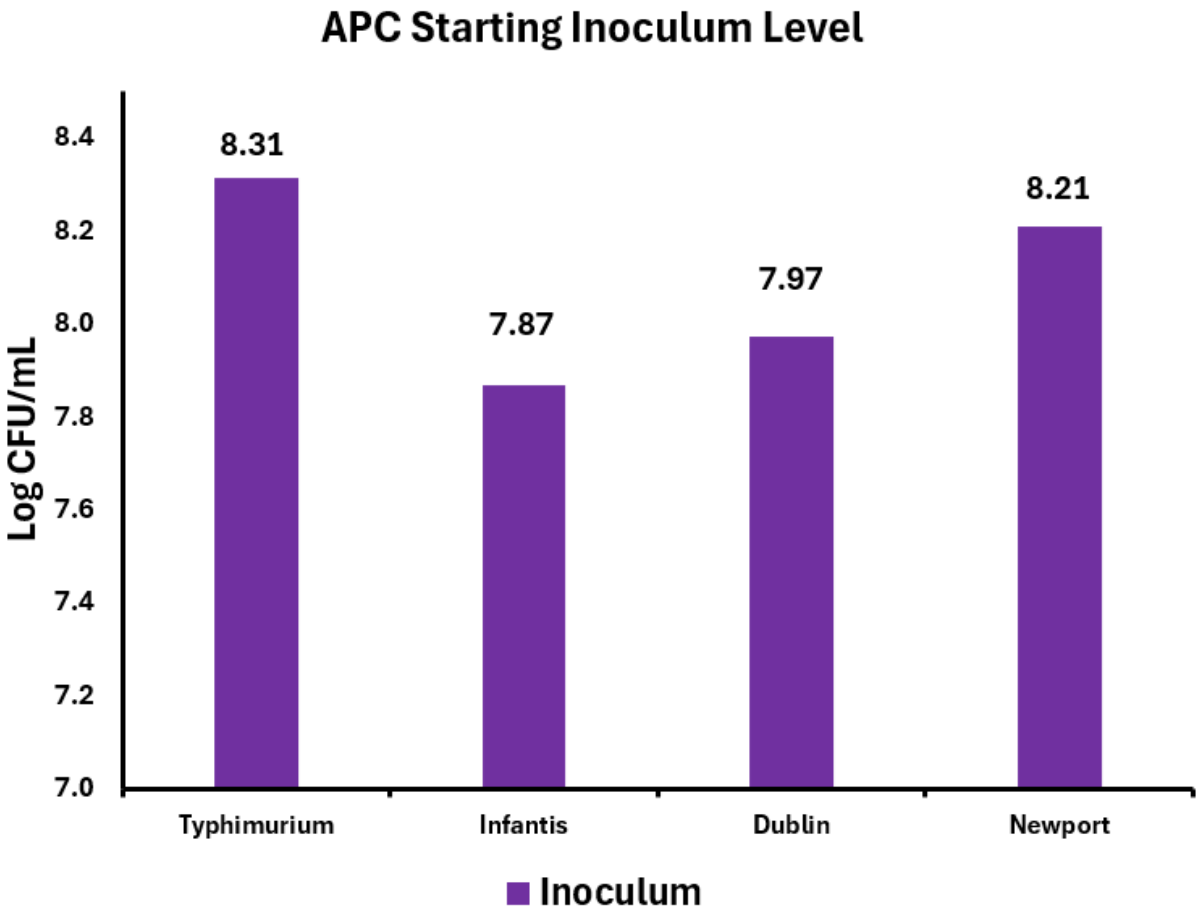

Figure 2.1 Estimated Log_{10} (CFU/mL) APC counts for each of the initial inoculums used to create treatment levels high, medium, and low for each serotype.

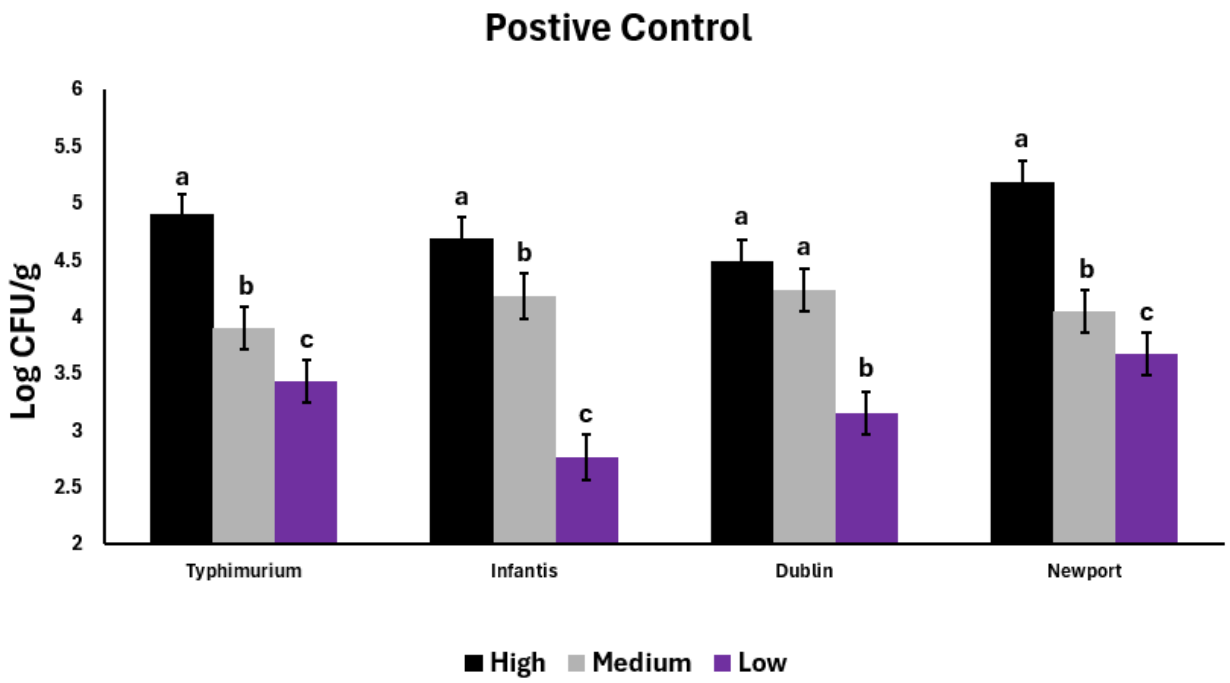

Figure 2.2 Estimated Log_{10} (CFU/g) for Positive Control samples for microbial levels post mixing for each serotype. Whiskers depict 95% confidence intervals. Different letters represent significant differences ($P<0.05$)

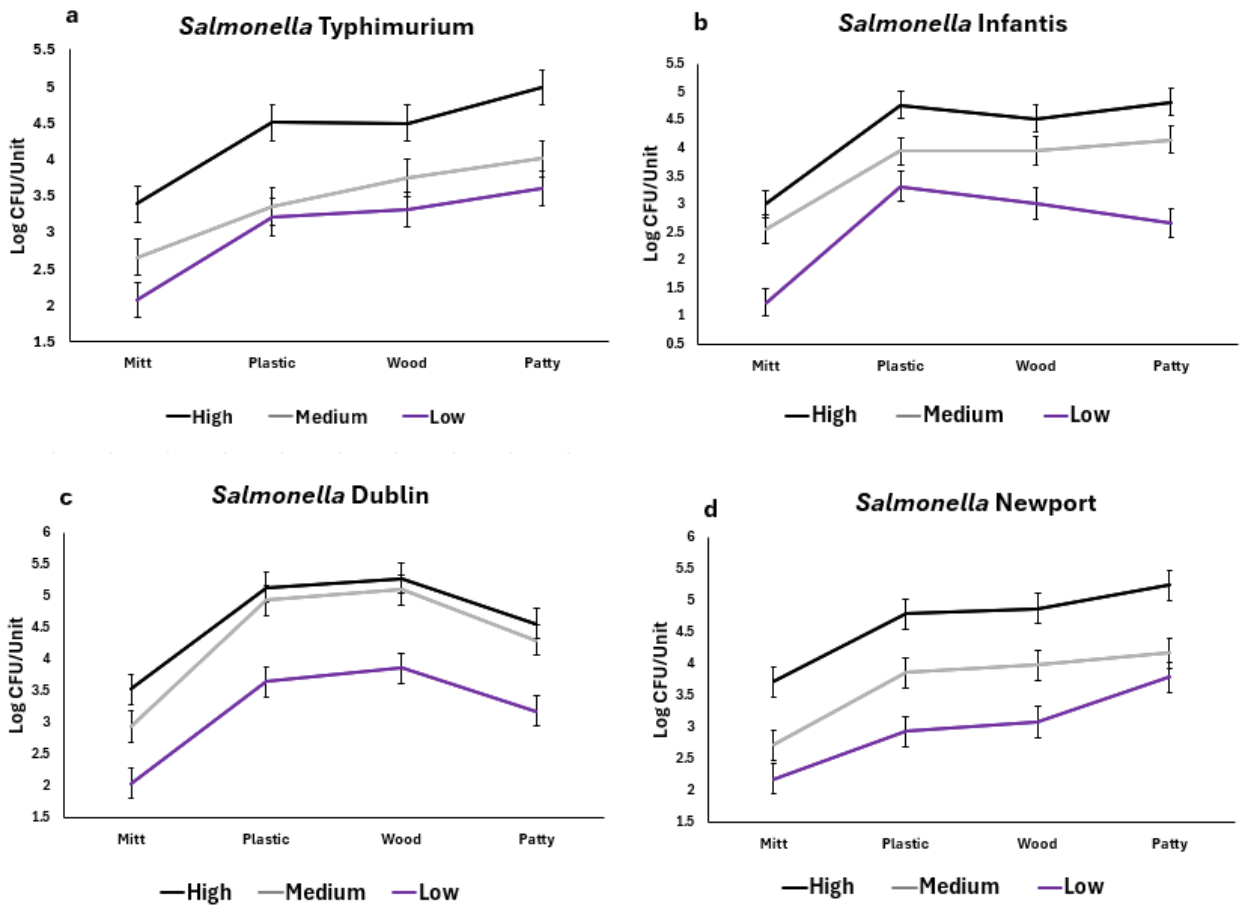

Figure 2.3 Log CFU/Unit recovery across surface type (mitt, plastic, wood, patty) for four *Salmonella* serotypes (**a**. Typhimurium, **b**. Infantis, **c**. Dublin, **d**. Newport) with three treatments (High, Medium, Low). Whiskers depict 95% confidence intervals ($P<0.05$). **a. For High and Low treatments:** Mitt was significantly different from Plastic, Wood, and Patty. Plastic and Wood were not significantly different from each other and were significantly different from Patty. Patty was significantly different from Mitt, Plastic, and Wood. **For Medium treatment:** Mitt was significantly different from Plastic, Wood, and Patty. Plastic was significantly different from Wood and Patty. Patty and Wood were not significantly different from each other and were significantly different from Mitt and Plastic. **b. For High and Medium treatments:** Mitt was significantly different from Plastic, Wood, and Patty. Plastic, Wood, and Patty were not significantly different from each other. **For Low treatment:** each surface type was significantly different from each other. **c. For all treatments:** Mitt was significantly different from Plastic, Wood, and Patty. Plastic and Wood were not significantly different from each other and were significantly different from Patty. Patty was significantly different from Mitt, Plastic, and Wood. **d. For all treatments:** Mitt was significantly different from Plastic, Wood, and Patty. Plastic and Wood were not significantly different from each other and were significantly different from Patty. Patty was significantly different from Mitt, Plastic, and Wood.

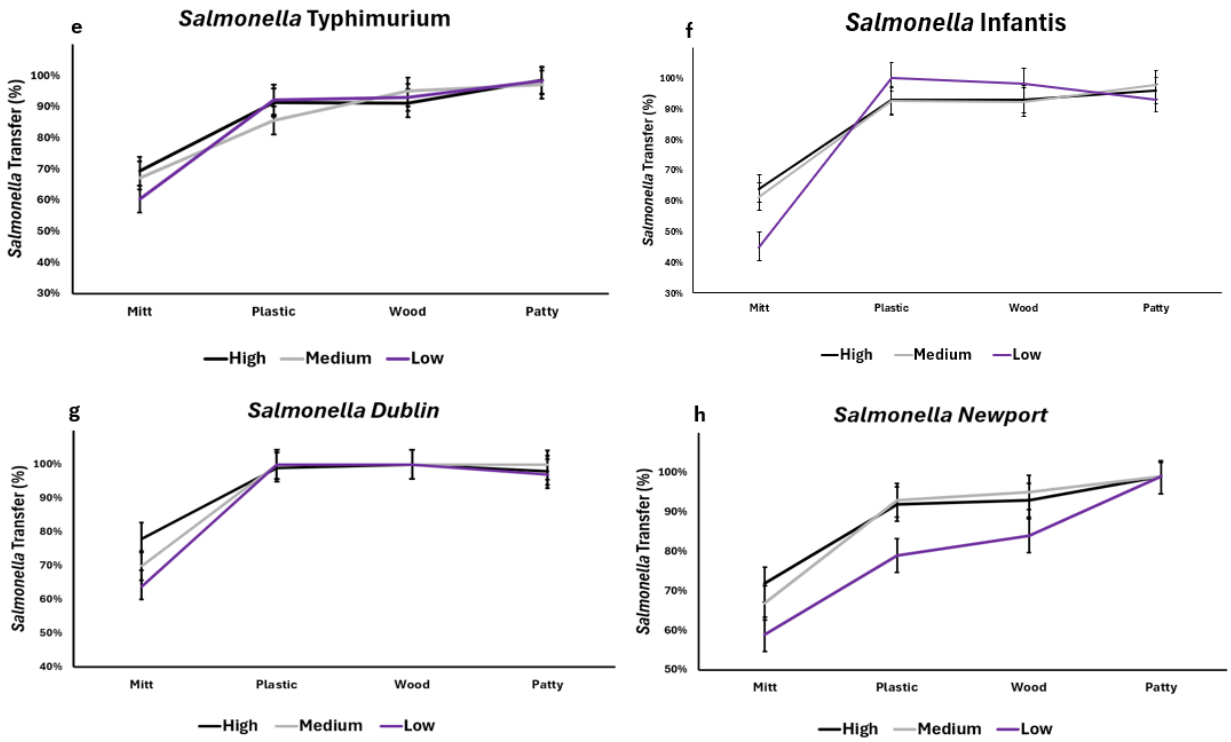

Figure 2.4 Estimated percent transfer rates across surface type (mitt, plastic, wood, patty) for four *Salmonella* serotypes (**e**. Typhimurium, **f**. Infantis, **g.** Dublin, **h.** Newport) with three treatments (High, Medium, Low). Whiskers depict 95% confidence intervals. ($P<0.05$). **e. For High and Low treatments:** Mitt was significantly different from Plastic, Wood, and Patty. Plastic and Wood were not significantly different from each other and were significantly different from Patty. Patty was significantly different from Mitt, Plastic, and Wood. **For Medium treatment:** Mitt was significantly different from Plastic, Wood, and Patty. Plastic was significantly different from Wood and Patty. Patty and Wood were not significantly different from each other and were significantly different from Mitt and Plastic. **f. For High and Medium treatments:** Mitt was significantly different from Plastic, Wood, and Patty. Plastic, Wood, and Patty were not significantly different from each other and were significantly different from Mitt. **For Low treatment:** Mitt was significantly different from Plastic, Wood, and Patty. Plastic was not significantly different from Wood and was significantly different from Patty. Patty was not significantly different from Wood and was significantly different from Mitt and Plastic. **g. For all treatments:** Mitt was significantly different from Plastic, Wood, and Patty. Plastic, Wood, and Patty were not significantly different from each other and were significantly different from Mitt. **h. For High and Low treatments:** Mitt was significantly different from Plastic, Wood, and Patty. Plastic and Wood were not significantly different from each other and were significantly different from Patty. Patty was significantly different from Mitt, Plastic, and Wood. **For Medium treatment:** Mitt was significantly different from Plastic, Wood, and Patty. Plastic was not significantly different from Wood and was significantly different from Patty. Patty was not significantly different from Wood and was significantly different from Mitt and Patty.

Serotype	Treatment	Surface	Log CFU/Unit	SEM	Lower mean 95% CI	Upper mean 95% CI
Typhimurium	High	Mitt	3.3784[a]	0.1264	3.1298	3.627
		Plastic	4.4998[b]	0.1264	4.2512	4.7484
		Wood	4.484[b]	0.1264	4.2354	4.7326
		Patty	4.974[c]	0.1217	4.7345	5.2135
	Medium	Mitt	2.6533[a]	0.1283	2.4008	2.9058
		Plastic	3.3511[b]	0.1283	3.0986	3.6036
		Wood	3.7411[c]	0.1283	3.4886	3.9936
		Patty	3.9978[c]	0.1283	3.7453	4.2503
	Low	Mitt	2.068[a]	0.1217	1.8285	2.3075
		Plastic	3.1946[b]	0.132	2.9351	3.4542
		Wood	3.305[b]	0.1217	3.0655	3.5445
		Patty	3.601[c]	0.1217	3.3615	3.8405
Infantis	High	Mitt	3.004[a]	0.1217	2.7645	3.24
		Plastic	4.775[b]	0.1217	4.5355	5.0145
		Wood	4.535[b]	0.1217	4.2955	4.7745
		Patty	4.829[b]	0.1217	4.5895	5.0685
	Medium	Mitt	2.5578[a]	0.1283	2.3053	2.8103
		Plastic	3.9456[b]	0.1283	3.6931	4.1981
		Wood	3.9533[b]	0.1283	3.7008	4.2058
		Patty	4.1567[b]	0.1283	3.9042	4.4092
	Low	Mitt	1.2444[a]	0.1283	0.9919	1.4969
		Plastic	3.3177[a]	0.134	3.0541	3.5813
		Wood	3.0102[a]	0.1408	2.7334	3.287
		Patty	2.6611[a]	0.1283	2.4086	2.9136
Dublin	High	Mitt	3.518[a]	0.1217	3.2785	3.7575
		Plastic	5.1255[b]	0.1217	4.8855	5.3645
		Wood	5.268[b]	0.1217	5.0285	5.5075
		Patty	4.56[c]	0.1217	4.3205	4.7995
	Medium	Mitt	2.932[a]	0.1217	2.6925	3.1715
		Plastic	4.928[b]	0.1217	4.6885	5.1675
		Wood	5.087[b]	0.1217	4.8475	5.3265
		Patty	4.298[c]	0.1217	4.0585	4.5375
	Low	Mitt	2.035[a]	0.1217	1.7955	2.2745
		Plastic	3.637[b]	0.1217	3.3975	3.8765
		Wood	3.858[b]	0.1217	3.6185	4.0975

		Patty	3.175[c]	0.1217	2.9355	3.4145
Newport	High	Mitt	3.718[a]	0.1217	3.4785	3.9575
		Plastic	4.793[b]	0.1217	4.5535	5.0325
		Wood	4.877[b]	0.1217	4.6375	5.1165
		Patty	5.24[c]	0.1217	5.0005	5.4795
	Medium	Mitt	2.719[a]	0.1217	2.4795	2.9585
		Plastic	3.854[b]	0.1217	3.6145	4.0935
		Wood	3.981[b]	0.1217	3.7415	4.2205
		Patty	4.169[c]	0.1217	3.9295	4.4085
	Low	Mitt	2.177[a]	0.1217	1.9375	2.4165
		Plastic	2.925[b]	0.1217	2.6855	3.1645
		Wood	3.079[b]	0.1217	2.8395	3.3185
		Patty	3.784[c]	0.1217	3.5445	4.0235

Table 2.1 Mean *Salmonella* recovery (Log CFU/Unit) from four serotypes (Typhimurium, Infantis, Dublin, and Newport) across three inoculation levels (high, medium, and low) and four surface types (mitt, plastic, wood, and patty). Values represent least squares means with corresponding standard error of the mean (SEM) and 95% confidence intervals (CI). Superscript letters (a-c) within each serotype and treatment indicate significant differences among surface types ($P<0.05$)

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