IMPACT OF TEMPORARY STORAGE ORIENTATION ON COMMENSAL BACTERIA AND SANITIZING PROGRAMS ON SURVIVABILITY OF *LISTERIA INNOCUA* ON FOOD CONTACT SURFACES IN RETAIL DELI SETTINGS

A Thesis

Presented to the

Faculty of the College of Graduate Studies and Research

Angelo State University

In Partial Fulfillment of the

Requirements for the Degree

MASTER OF SCIENCE

by

JADE ELAINE ATKINSON

December 2019

Major: Animal Science

IMPACT OF TEMPORARY STORAGE ORIENTATION ON COMMENSAL BACTERIA AND SANITIZING PROGRAMS ON SURVIVABILITY OF *LISTERIA INNOCUA* ON FOOD CONTACT SURFACES IN RETAIL DELI SETTINGS

by

JADE ELAINE ATKINSON

APPROVED:

Dr. Loree Branham

Dr. John Kellermeier

Dr. Chase Runyan

Dr. Trey Smith

December 2019

APPROVED:

Dr. Micheal W. Salisbury
Dean, College of Graduate Studies and Research

ACKNOWLEDGEMENTS

I would like to start off by thanking my committee for their time and patience throughout this whole process. Dr. Branham, Dr. Kellermeier, Dr. Runyan, and Dr, Smith, thank you for your support and guidance in helping me to complete this final stage of my degree. I would also like to thank Alicia Curtis for helping me in the lab. Your help was so appreciated.

To Dr. Branham, as an undergrad I had always looked up to you as a mentor and a confidante. Your classes are what helped me in figuring out what I wanted to do as a career. Thank you so much for taking me under your wing and being patient with me, teaching me, guiding me, and helping me grow into the person I am today.

To my mom, thank you. Thank you for raising me to be who I am today. You raised me to believe I can do anything and everything. You have given me everything in order for me to achieve anything. Thank you for always being there when I needed you. Thank you for always supporting me, financially and emotionally. Thank you for being my shoulder to cry on. Thank you for always being the person I can go to for anything. You are my best friend and I am so thankful God chose me to be your daughter. I really lucked out at having the best mom in the world.

ABSTRACT

The aim of this study was to evaluate the impact of temporary storage orientation on commensal bacterial populations and sanitizing programs on survivability of *Listeria innocua* on food contact surfaces in retail deli settings. For trial 1, when looking only at salami, the face orientation resulted in higher contamination levels compared to the butt ($P \leq 0.05$). When looking only at turkey, there was no significant difference in microbial growth populations of both orientations ($P \geq 0.05$). For trial 2, When looking at both turkey and salami samples, there was no significant difference in microbial growth levels after the cleaning and sanitizing and sanitizing only treatments were applied ($P \geq 0.05$) However, there was at least a 3-log reduction of the turkey and a 5-log reduction of the salami compared to the control. Overall, the current study concluded that the effect of the treatment orientation of ready-to-eat (RTE) meat on microbial contamination varies depending on meat product type. Additionally, both cleaning programs were equally as effective in reducing microbial growth on food contact surfaces in retail deli settings.

TABLE OF CONTENTS

ACKNOWLEDGEMENTS iii
ABSTRACT iv
TABLE OF CONTENTS v
LIST OF TABLES vi
LIST OF FIGURES vii
INTRODUCTION 1
LITERATURE REVIEW 3
Cleaning and Sanitation Practices in Retail Deli's 3
Listeria monocytogenes 3
Surrogates 4
Listeria innocua 5
Pseudomonas spp. 6
Aerobic Plate Count 7
Polyethylene Board as a Food Contact Surface 7
MATERIALS AND METHODS 8
Sample Collection 8
Trial 1 8
Trial 2 9
Trial 3 10
Statistical Analysis 11
RESULTS AND DISCUSSION 12
Trial 1 12
Trial 2 16
Trial 3 21
CONCLUSION 25
LITERATURE CITED 26
BIOGRAPHY 35

LIST OF TABLES

Tabe 1. Least Squares Means (LSMeans) of Aerobic Plate Count (Log_{10} CFU/100 cm^2) obtained from the surface of a cutting board after contact with the butt and face of turkey (n = 64) and salami (n = 64) 16

Table 2. Least Squares Means (LSMeans) of Aerobic Plate Count (Log_{10} CFU/100 cm^2) obtained from the surface of a cutting board after contact with turkey (n = 48) and salami (n = 48) inoculated with *Listeria innocua* following either no treatment (control = 8), cleaning and sanitizing (n = 20) or sanitizing only (n = 20)....................21

Table 3. Prevelence of generic *Listeria* spp. samples obtained from the purge of commercially processed deli loaves and plated onto 3M Environmental *Listeria* petrifilm ...24

LIST OF FIGURES

Figure 1. Percent of samples positive for any aerobic bacterial growth obtained from the surface of a cutting board after contact with the butt or face of turkey (n = 64) and salami (n = 64) loaves 14

Figure 2. Percent of samples positive for *Listeria innocua* obtained from the surface of a cutting board after contact with inoculated turkey (n = 48) and salami (n = 48) following either no treatment (control = 8), cleaning and sanitizing (n = 20) or sanitizing only (n = 20) 19

INTRODUCTION

Microbial growth due to improper cleaning and sanitizing can lead to cross contamination on food contact surfaces and an unsafe food processing environment (Dunsmore, 1981; Somers and Wong, 2004). Krysinski et al. (1991), and Somers and Wong (2004), conclude the most effective cleaning method used in food processing environments to eliminate microbial growth includes using a detergent, a rinse, followed by a sanitizer. In retail deli settings, it is not uncommon to see workers cleaning with only a sanitizing wipe. Some studies suggest that sanitizing wipes are not as effective as the cleaning, rinsing, and sanitizing methods because they don't remove all the food soil on food contact surfaces, thus resulting in microbial growth (Bolton et al., 2013; Gibson et al., 2012). Studies have recognized microbial contamination as the main cause of ready-to-eat (RTE) spoilage and illness (Perez-Rodriguez et al., 2007; Vorst et al., 2006a; Vorst et al., 2006b). Contamination of RTE meats can happen at the early stages of handling at the retail level (Perez-Rodriguez et al., 2007; Vorst et al., 2006a; Vorst et al., 2006b). During the retail preparation of RTE meat products, if contamination with pathogenic organisms occurs, this can cause a foodborne illness outbreak amongst consumers (EFSA, European Food Safety Authority, 2007). Olsen et al. (2000) found that each year from 1993 to 1997, there was a total of 2,671 foodborne illnesses (FBI) in the United States caused by improper food preparation practices. Hence, the Food and Drug Administration (FDA) enforces proper cleaning and sanitizing in retail deli's to help prevent or lessen the chances of cross-contamination from occurring (FDA, 2017).

____

Journal of Animal Science

The objectives of this project included: 1) determine the impact product orientation during storage periods has on microbial growth of residue left on contact surfaces, 2) determine if single step sanitizing in the form of a sanitation cloth is as effective as cleaning and sanitizing, and 3) assessing *Listeria* spp. presence in RTE purge of commercially prepared luncheon meat.

LITERATURE REVIEW

Cleaning and Sanitation Practices in Retail Deli's

The growth of food preparation and sales has increased the need for proper cleaning and sanitizing practices (Marriot et al., 2018). According to the FDA, the proper cleaning method is using a detergent, rinse, followed by a sanitizer (FDA, 2017). Aside from the rinse and sanitizer, Dunsmore (1981) stated that detergent was the most important factor in controlling or eliminating the majority of microbial growth. Although the cleaning, rinsing, and sanitizing method is recommended, sanitizer infused wipes are commonly used to clean food contact surfaces as well. Because the wipes are easy to use and disposable, they can reduce the amount of time it takes to clean a food contact surface. However, because of its short contact time, it is possible that it might not remove all food residue from the contact surface (Bolton et al., 2013; Gibson et al., 2012). Studies show microorganisms become increasingly more difficult to remove once it attaches to a surface (Krysinski et al., 1991). *Listeria monocytogenes*, for example, can adapt to a variety of environments, which make it harder to eliminate (Donnelly, 2001). A study conducted by Somers and Wong (2004) found that *L. monocytgenes* biofilms were greatly reduced on several different surfaces by using a cleaning and sanitizing programs combination. These are just a few examples of how cleaning and sanitizing is considered the most effective cleaning method.

Listeria monocytogenes

Microbial activity is responsible for the deterioration of some foods and can cause a decline in food quality and overall safety for consumers (Dinev et al., 2018). According to Rahman and Kang (2009), consumers have become more concerned with pathogenic microorganisms because of foodborne disease outbreaks. *Listeria monocytogenes* is a Gram-positive bacteria that causes major concern within the food industry (Farver and Peterkin,

1991). This pathogen can be found in a variety of places including food-processing environments because of its universal nature to adapt (Donnelly, 2001). *Listeria monocytogenes* is not only a public health concern due to the amount of illnesses and hospitalizations it causes, but it can be an economic issue due to food recalls as well (Gandhi and Chikindas, 2007). *Listeria* is the main pathogen of concern associated with ready-to-eat (RTE) meat products (Lianou and Sofos, 2007). Between the years 2000 and 2005, a total of 91 Listeriosis outbreaks occurred prompting the USDA to develop improved methods of testing and packaging to control *Listeria* in delicatessen meats (U.S. Food Safety and Inspection Service, 2003). A method to control *Listeria* is through multiple hurdle technologies. Multiple hurdle technologies are factors such as chilling and freezing which aid in the preservation of food and elimination of spoilage microorganisms (Leistner, 1994). An example includes a study conducted by Mertz et al. (2015), which found that applying moist heat with a sanitizer eliminated *Listeria* on stainless steel surfaces. *Listeria* is one of the only pathogens capable of growing at a temperature below 5°C (James et al., 2016). If temperature abuse occurs, *Listeria* could grow to infectious levels (Loncarevic et al., 1996). Although sanitation processes have been improved throughout the years, *L. monocytogenes* outbreaks continue to occur. Because of this, further research must take place to better improve sanitation procedures so as to lessen the chances of such outbreaks from occurring in the future (Ndahetuye et al., 2012).

Surrogates

Surrogates are organisms, usually bacteria, used in studies assessing intervention impacts on pathogenic bacteria by studying the effects of applied treatments without compromising the safety of the workers and the safety of the processing environment (Busta et al., 2003). Inoculation of food is one example of how a surrogate can be used to test the

effectiveness of different equipment or experimental strategies (Rodriguez et al., 2006; Slade, 2003). Busta et al. (2003) studied the use of indicator and surrogate microorganisms in fresh cut produce. The study introduced the idea that because produce does not have a processing elimination step to remove pathogens, surrogates could be inoculated onto produce or equipment surfaces as test organisms to determine the effectiveness of certain cleaning programs.

When selecting surrogates, certain qualifications such as being non-pathogenic, easy to prepare, and having similar behaviors to that of the target organism are desired (Center for Food Safety and Applied Nutrition and Food and Drug Administration, 2001). These criteria are most ideal when selecting surrogates because they help to make inoculation safe and sterile (Busta et al., 2003). When choosing surrogates specifically for food, it is important to look for the pathogenic bacteria most associated with that food product. For example, when working with deli meats, the main pathogen of interest would be *L. monocytogenes.* Strains of *L. innocua* would serve as the non-pathogenic surrogate of *L. monocytogenes* (Busta et al., 2003).

Listeria innocua

Listeria innocua is a non-hemolytic, non-pathogenic strain of *Listeria* (Favaro et al., 2014). It is used as a means of inoculation because it is most closely biologically related to *L. monocytogenes* (Duh and Schaffner 1992; Favaro et al., 2014). *Listeria innocua* and *L. monocytogenes* show most of the same characteristics, share the same environment and can be associated with the same food products. *L. innocua* can grow at a faster rate and at a higher population on enrichment mediums than *L. monocytogenes*, which makes *L. innocua a* good surrogate for *L. monocytogenes* (Fatima et al., 2009; Curiale and Lewus, 1994). A study conducted by Rod et al. (2012) wanted to observe the changes in oxidation and color by

using *L. innocua* as a surrogate for *L. monocytogenes* because of their similarities with reactive oxygenated species. Rod et al. (2012) inoculated 25 µl of a decimal dilution of 10^8 CFU/ml on the upward facing side of the meat samples. The samples were placed into sealed polymer bags and treated by an indirect plasma treatment. The study showed that the indirect plasma treatment reduced *L. innocua* on RTE meat products and could therefore reduce *L. monocytogenes* if the situation arose. Another study conducted by Bourion and Cerf (1996) compared different sanitizing programs against biofilm formation of both *Listeria innocua* and *Pseudomonas aeruginosa.* Bourion and Cerf (1996) found that *Pseudomonas* biofilm protected *Listeria* against sanitizers therefore reducing their effectiveness. These are just a few examples of how *L. innocua* could act as an effective surrogate for *L. monocytogenes*.

***Pseudomonas* spp.**

Pseudomonads are cold thriving spoilage organisms most commonly found in meat and dairy products (Rajmohan et al., 2002). These spoilers are a threat to the food service industry because of their ability to form biofilms on food and food contact surfaces. Biofilms are a type of bacterial growth, which can be very difficult to eliminate (Joseph et al., 2001). Bacterial growth on food can have a significant impact on economic losses (Gram et al., 2002). Biofilm cells have adapted to evade normal cleaning and sanitizing procedures, which could be a source of cross-contamination of food (Joseph et al., 2001). For example, evidence shows that *Pseudomonas aeruginosa* can adapt to quaternary ammonium compounds (QACs), if exposed for long periods of time, making it harder to eliminate off of food contact surfaces (Langsrud and Sundheim, 1997). By improving the preventative methods for biofilm formation, this could reduce the amount of sanitation chemicals needed and could therefore lower the cost for producing a safe and wholesome product (Joseph et al., 2001).

Aerobic Plate Count

Aerobic plate count (APC) is one of the most common tests used to indicate microbial quality of food (Food Standards, 2001). It is used for evaluating food products and their related environments for freshness and hygienic practices (Kodaka et al., 2005). Depending on the type of food being tested, the results of the APC can vary which is useful in observing and comparing its bacterial development over time (Food Standards, 2001). Aerobic plate count media grows colonies indicating either acceptable or unacceptable quality. A high APC indicates the food has been either mishandled or is poor quality (Wagner, 2008). Poor quality of food can suggest growth of spoilage microorganisms (Sperber and Doyle, 2008). Methods such as APC provide the information needed to allow researchers the ability to improve the quality and safety of food for consumers.

Polyethylene Board as a Food Contact Surface

Cutting boards are common tools utilized in the food service industry. Their material can range anywhere from wood, bamboo, to polyethylene (Carpentier, 1997). Polyethylene is the most common cutting board used because of its nonporous material and its inability to absorb liquid, which allows for proper cleaning and sanitizing (Miller et al., 1996). A study was conducted by Yang et al. (2008) that tested for the effectiveness of different sanitizing programs against *Listeria monocytogenes* biofilms on both rough and soft polyethylene cutting board surfaces. Their results showed that *L. monocytogenes* cells can survive on cutting boards for up to 6 days when the boards are not cleaned, but levels are significantly reduced when sanitized. It is recommended to sanitize immediately after each use to avoid biofilm formation.

MATERIALS AND METHODS

Sample Collection

Sixteen vacuumed packaged, fully cooked mesquite turkey breast deli loaves and 16 vacuumed packaged, fully cooked hard salami deli loaves were obtained from a federally inspected facility and stored at 4°C at the Angelo State University's Food Safety Product Development Laboratory. Of those total loaves, 8 of both meats were randomly assigned to trial 1 and the other 8 were assigned to trial 2. The contact surfaces utilized in both trial 1 and trial 2 were ultra high molecular weight polyethylene (UHMWPE) cutting boards. Before use, the cutting boards were gridded off into 10 x 10 cm size squares for a total surface area of 100 cm^2. After gridding, the contact surfaces were cleaned and disinfected with a food grade antimicrobial detergent and sanitizer.

Trial 1

The first trial evaluated the impact of butt (rounded surface) vs. face (fresh cut flat face surface) orientation of the product during the interim time between slicing and repackaging of the meat loaf in a deli setting. The average surface area of the face of the turkey deli loaves was 35.01 $cm^{2,}$ and the average surface area of the butt making contact with the cutting board was 18.72 cm^2. The average surface area of the face of the salami deli loaves was 15.75 cm^2 and the average surface area of the butt making contact with the cutting board was 1.45 cm^2. A total of 64 pieces of each meat were randomly assigned to one of two treatments. In treatment 1, 32 samples of salami and 32 samples of turkey were laid with the product sliced surface down to have contact with the countertop. In treatment 2, 32 samples of salami and 32 samples of turkey were laid with the product butt portion in contact with the countertop. The product was allowed to sit at room temperature for 20 minutes before it was removed. Once removed, the contact surface sat undisturbed for another 20 minutes before the 100 cm^2 surface area was swabbed using a 3M pre-hydrated quickswab.

Serial dilutions using buffered peptone water (BPW) were performed, and 1 mL was taken from each serial dilution and plated in duplicate using a 3M Aerobic Plate Count petrifilm (3M ™, Maplewood, MN) in accordance with 3M procedures. The plates were incubated for 48 ± 3 hours at 35 ± 1°C. The 3M AOAC approved protocol #990.12 was followed. Typical Aerobic colonies are red in color and circular in shape. Populations were counted using the 3M standard countable limit range of 25-250 and entered into an excel spreadsheet. The treatment orientation that resulted in the highest microbial load was utilized in trial 2.

Trial 2

Trial 2 evaluated the impact of two different cleaning programs that occur in a retail deli setting, along with an untreated control. A total of 40 pieces of each meat were randomly assigned to one of two treatments. Eight pieces of each meat were assigned to the control. Treatment 1 included cleaning and sanitizing, while treatment 2 focused on sanitation alone. The contact surfaced was gridded off into 100 cm^2 squares and disinfected with Oasis 146 Multi-Quat Sanitizer (Alkyl Dimethyl benzyl ammonium chloride, octyl decyl dimethyl ammonium chlorie, dodecyl dimethyl ammonium chloride, dioctyl dimethyl ammonium chloride) (Ecolab, St. Paul, MN) before and after treatments and sampling. The meat was inoculated with a mixture of two strains of *Listeria innocua* (ATCC #51742 and #33090). In order to inoculate, a freeze dried vial of each strain from the American Type Culture Collection was hydrated with sterile Brain Heart Infusion broth (BHI). One mL of suspended culture was placed into 6 mL of sterile BHI and incubated for 24 hours at 37°C. Each day of trial 2, one mL of previously grown culture was transferred to a sterile 9 mL tube of BHI and incubated for 24 hours at 37°C. One mL of both ATCC #51742 and 33090 was added to 198 mL of sterile Tryptic Soy Broth and mixed well. This served as the inoculation culture for trial 2. The straight inoculation culture was plated for reference purposes in duplicate and

recorded as 6.74 $\log_{10}$ CFU/mL. All meat inoculation was performed under a BSL 2 certified hood. One mL of the inoculate was pipetted onto the meat surface and spread using a sterile hockey stick and allowed to attach for 1 h. The meats were then placed inoculated surface down on various cutting board sections. The meat samples were allowed to sit at a room temperature of 20-25°C for 20 min before they were removed. Once removed, the contact surface remained undisturbed for another 20 min in order to allow bacteria to adhere. A 3M Quick Swab was then used to swab the control sample areas, while the other sample areas underwent their randomly assigned treatments. For the cleaning and sanitizing treatment, 0.8 mL of Pantastic (water, coco DEA, sodium lauryl ether ethoxy sulfate, sodium lauryl sulfate, sodium chloride) (Ecolab, St. Paul, MN) per 1 L of water was used as the antimicrobial detergent. After spraying the detergent, a sterile food grade sponge was use to scrub the 100 cm^2 area. After scrubbing, a rinse with sterile water was applied to remove excess detergent. Immediately after, one Eco-Wipe Duo sanitizing wipe (Didecyl dimethyl ammonium chloride, dimethyl benzyle ammonium chloride) (Ecolab, St. Paul, MN) was used per sample area as the chemical sanitizer to disinfect. Treatment two underwent just the sanitizing step using the same type sanitizing wipe as treatment 1. The treated areas were allowed to dry for 10 min before a 3M Quick Swab was used to swab the sample area of 100 cm^2. Serial dilutions were plated onto 3M aerobic plate count petrifilm. The plates were incubated at 35 ± 1°C for 48 ± 3 hours. Plates were enumerated and data was entered into Excel.

Trial 3

Trial 3 utilized 1-10 mL of purge liquid from the original vacuum package bags containing the deli loaves to look for generic *Listeria* spp. presence. In the instance where 1-10 mL of purge was unattainable, the bag was swabbed to attain a sample. Two mL of purge

was added to BPW tubes and vortexed. Three mL was then taken and plated onto 3M Environmental *Listeria* petrifilm following 3M AOAC approved protocol PTM #030601. The plates were then incubated at 35 ± 1°C for 28 ± 2 hours and evaluated for growth. Presumptive positive *Listeria* spp. colonies appear to be small, smooth, circular and red-violet in color.

Statistical Analysis

Descriptive statistics were generated using the frequency procedure of SAS version 9.1.3. Microbial loads were transformed to a log base 10 and imported into SAS for analysis. Analysis of variance was utilized to determine differences in bacterial populations using the Mixed Procedure of SAS. Chi Square Analysis was utilized to evaluate differences in frequency of positive versus negative samples for trials 1. A predetermined alpha level of ≤ 0.05 was utilized.

RESULTS AND DISCUSSION

Trial 1

The objective of trial 1 was to determine if there was a difference in microbial contamination levels on a food contact surface that came in contact with the butt and face of moist and dry meat products. Out of 64 total salami associated samples evaluated, 37.50% of the face oriented salami samples (12 of 32) tested positive for any microbial growth compared to 12.50% of the butt oriented salami samples (4 or 32) that tested positive for any microbial growth ($P \leq 0.05$). Whereas, out of 64 turkey associated samples evaluated, 9.38% of the butt oriented turkey samples (3 of 32) testing positive for any microbial growth compared to 0% of the face oriented turkey samples (0 of 32) that tested positive for any microbial growth (Figure 1). The Chi Square Analysis was not reported for turkey samples due to low frequency of positive turkey associated samples.

When comparing microbial contamination levels on the food contact surface, there was a statistically significant interaction between sample orientation and meat type ($P = 0.01$). Microbial growth populations were higher on the face oriented salami samples at 0.87 $\log_{10}$ CFU/100 cm^2 compared to 0.32 $\log_{10}$ CFU/100 cm^2 of the butt oriented salami samples ($P \leq 0.05$). There was no statistical difference in microbial growth populations between the butt oriented turkey samples of 0.19 $\log_{10}$ CFU/100 cm^2 compared to 0 $\log_{10}$ CFU/100 cm^2 of the face oriented turkey samples ($P \geq 0.05$) When comparing the different meat types within one orientation type, there was a significant difference between the face orientation of turkey (0 $\log_{10}$ CFU/100 cm^2) and the face orientation of salami (0.87 $\log_{10}$ CFU/100 cm^2)($P \leq 0.05$). However, there was no significant difference between the butt orientation of turkey (0.19 $\log_{10}$ CFU/100 cm^2) and the butt orientation of salami (0.32 $\log_{10}$ CFU/100 cm^2) ($P \geq 0.05$) (Table 1).

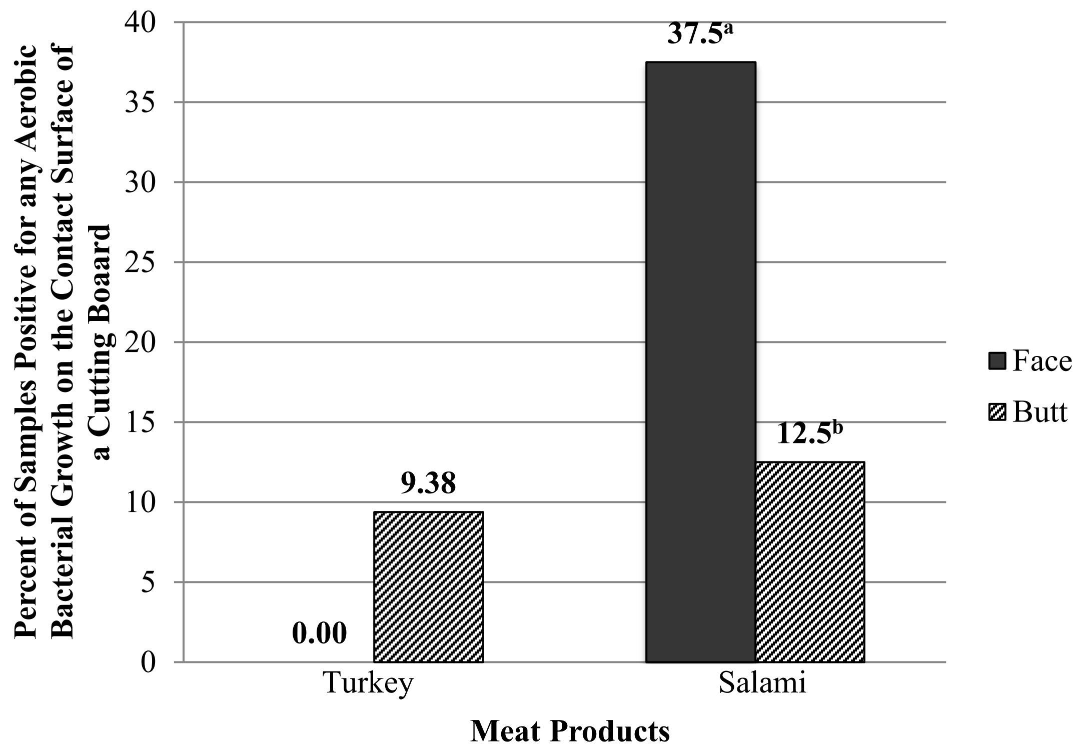

Figure 1. Percent of samples positive for any aerobic bacterial growth obtained from the surface of a cutting board after contact with the butt or face of turkey (n = 64) and salami (n = 64) loaves.

[a,b] Values within a meat type with different superscripts differ ($P \leq 0.05$).

* Chi Square Analysis not run on turkey due to low frequency of positive turkey associated samples

Table 1. Least Squares Means (LSMeans) of Aerobic Plate Count (Log_{10} CFU/100 cm^2) obtained from the surface of a cutting board after contact with the butt and face of turkey (n = 64) and salami (n = 64)

	Turkey (n = 64)		Salami (n = 64)	
Treatment	Log_{10} CFU/100 cm^2	SE*	Log_{10} CFU/100 cm^2	SE
Face	0.00^{a}	0.14	0.87^{bx}	0.14
Butt	0.19	0.14	0.32^{y}	0.14

^{a,b} Values within a treatment type with different superscripts differ ($P \leq 0.05$).
^{x,y} Values within a meat type with different superscripts differ ($P \leq 0.05$).
* SE = Standard Error

A study conducted by Vorst et al. (2006a) looked at the transfer of *Listeria* during mechanical slicing of turkey and salami. The study showed that the fat content of salami served as a “favorable medium” for *Listeria*, whereas the moisture content of the turkey had a “washing effect”. The data from the current study suggests that the salami had a higher microbial count than turkey, which may be due to the salami’s fat content or turkey’s moisture content. A similar study conducted by Lin et al. (2006) looked at cross contamination of salami and turkey by *Listeria* inoculation. The study showed more positive *L. monocytogenes* samples in turkey than salami, which contained lactate and diacetate, two common microbial inhibitors used in meat products (Mbandi and Shelef, 2000). Although the data from the current study indicates salami having a higher microbial count, both meats did contain microbial inhibitors. If both meats were to have been made without microbial inhibitors, the initial microbial count could have possibly been higher.

The initial hypothesis of this trial was that the face of turkey would contain the highest microbial count. However, data revealed that both the face and butt of salami resulted in higher contamination of the contact surface than the face and butt of turkey. One reason for this may be due to the antimicrobial agents added to the turkey. One ingredient in the turkey deli loaves was sodium nitrite. Sodium nitrite has been shown to improve product color, shelf life, and control or prevent the growth of pathogenic bacteria (Sindelar and Milkowski, 2011). Salami loaves also contained antimicrobial inhibitors such as sodium nitrite, butylated hydroxyanisole (BHA), and butylated hydroxytoluene (BHT). Butylated hydroxyanisole and BHT are antioxidants added in meat which help prevent spoilage, improve quality, and maintain nutritional value in food (Kumar et al., 2015). In the current study, both turkey and salami contained antimicrobial inhibitors. However, it is unsure if

inhibitors impacted results. When referring to the previous study by Vorst et al. (2006a), the turkey's moisture content may contribute to its low microbial count by essentially washing away any form of microbial growth. Additionally, the fat content of the salami may have acted as an attractant for microbial growth to cling onto. Research such as Lin et al. (2006), and USDA, FSIS (2012) provide information on the moisture and fat content of meats and how it may impact microbial growth going forward. This trial was used as a means of determining the safest way to temporarily place deli loaves on food contact surfaces in a retail deli setting. Results varied depending on meat type and placement orientation.

Trial 2

The objective of trial 2 was to determine if there were differences in level of residual microbial contamination when comparing two different cleaning programs on food contact surfaces after contamination with inoculated meat. Out of 16 total samples evaluated, 100% of the control samples for both turkey (8 of 8) and salami (8 of 8) tested positive for microbial growth and were used as a comparison to the two cleaning and sanitizing treatments. Out of 40 total salami associated samples evaluated, there were more sanitizing only samples (2 of 20) testing positive with 10.00% of samples testing positive for any microbial growth compared to 5.00% of the cleaning and sanitizing program samples (1 of 20). Out of 40 total turkey associated samples evaluated, there were more sanitizing only samples (10 of 20) testing positive with 50.00% of samples testing positive for any microbial growth compared to 35.00% of the cleaning and sanitizing program samples (7 of 20) (Figure 2). The Chi Square Analysis was not reported due to low frequency of positive turkey and salami associated samples. This information is important because it shows how effective both cleaning programs were when compared to the untreated control. It is also important for future studies, which could help to identify more effective methods of cleaning.

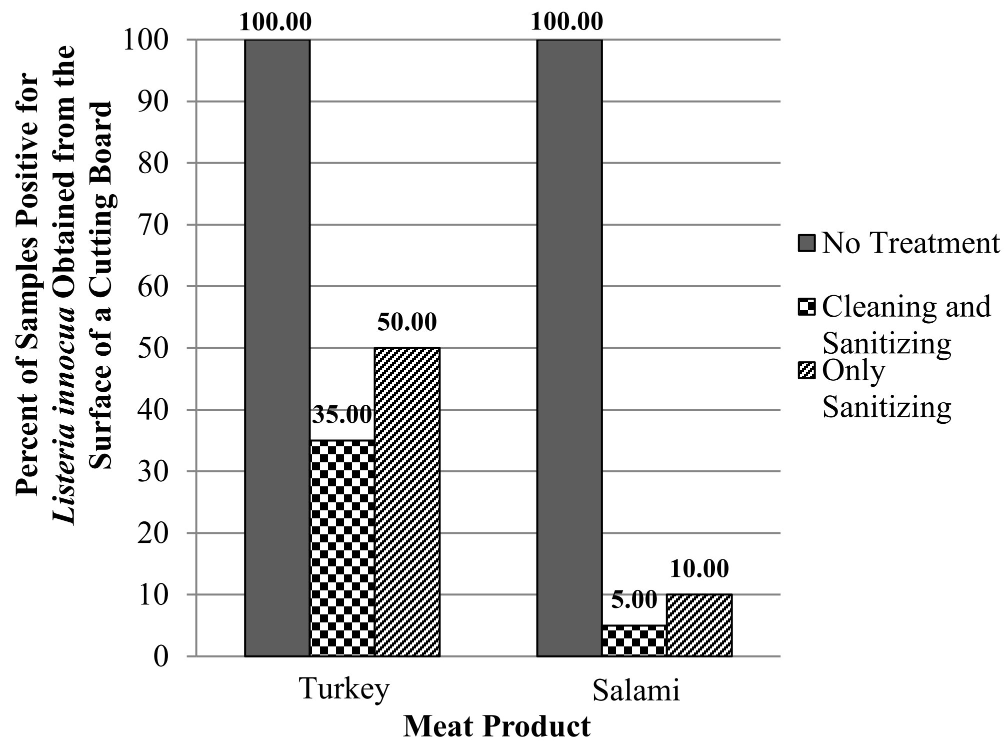

Figure 2. Percent of samples positive for *Listeria innocua* obtained from the surface of a cutting board after contact with inoculated turkey (n = 48) and salami (n = 48) following either no treatment (control = 8), cleaning and sanitizing (n = 20) or sanitizing only (n = 20).

When comparing *L. innocua* contamination levels on the surface of the associated cutting board, within each meat type, there was a statistically significant interaction between the cleaning programs and meat products ($P = 0.004$). When comparing the controls, there was no significant difference between turkey 4.70 log_{10} CFU/100 cm^2 and salami 5.44 log_{10} CFU/100 cm^2 ($P \geq 0.05$). When comparing microbial growth populations of turkey samples, there was no significant difference between the cleaning and sanitizing program of 0.87 log_{10} CFU/100 cm^2 and the sanitizing only program of 1.21 log_{10} CFU/100 cm^2 ($P \geq 0.05$). However, both treatment types showed a significant reduction of at least 3.5-log compared to the control. When comparing microbial growth populations of salami, there was no significant difference between the cleaning and sanitizing program of 0.05 log_{10} CFU/100 cm^2 and the sanitizing only program of 0.19 log_{10} CFU/100 cm^2 ($P \geq 0.05$). However, both treatments were significantly lower than the control, showing at least a 5-log reduction compared to the control. For a study of this size, seeing a 3 and 5 log reduction is important in determining effectiveness of cleaning programs when compared to the control. When comparing the different meat types within one treatment type, there was a significant difference between the cleaning and sanitizing program of turkey (0.87 log_{10} CFU/100 cm^2) and the cleaning and sanitizing program of salami (0.05 log_{10} CFU/100 cm^2) ($P \leq 0.05$). There was also a significant difference between the sanitizing only program of salami (0.19 log_{10} CFU/100 cm^2) and the sanitizing only program of turkey (1.21 log_{10} CFU/100 cm^2) ($P \leq 0.05$) (Table 2). This information is important because although it shows no difference when comparing the values within a treatment type, it does show a significant difference of the values within a meat type. This information is beneficial because it could help future studies to create different cleaning methods that are associated with specific meat types.

Table 2. Least Squares Means (LSMeans) of Aerobic Plate Count (Log_{10} CFU/100 cm^2) obtained from the surface of a cutting board after contact with turkey (n = 48) and salami (n = 48) inoculated with Listeria innocua following either no treatment (control = 8), cleaning and sanitizing (n = 20), or sanitizing only (n = 20).

	Turkey (n = 48)		Salami (n = 48)	
Treatment	Log_{10} CFU/100 cm^2	SE*	Log_{10} CFU/100 cm^2	SE
Control (n=8)	4.70^{x}	0.32	5.44^{x}	0.32
Cleaning and Sanitizing (n=20)	0.87^{ay}	0.20	0.05^{by}	0.20
Sanitation Only (n=20)	1.21^{ay}	0.20	0.19^{by}	0.20

^{a,b} Values within a treatment type with different superscripts differ ($P \leq 0.05$)

^{x,y} Values within a meat type with different superscripts differ ($P \leq 0.05$)

* SE = Standard Error

One of the most effective ways to remove biofilm formation is by cleaning, rinsing, and sanitizing (Somers and Wong, 2004). Krysinski et al. (1991) noted that detergent is used to remove food soils while the sanitizer disables bacteria that are left behind after the initial cleaning. Krysinski et al. (1991) conducted a study that looked at the effectiveness of cleaners and sanitizer on *L. monocytogenes* attached to food contact surfaces. The study found that the detergents and sanitizers were most effective when used together. When tested separately, the chemical detergents and sanitizers were only effective in removing some organisms on certain surfaces. When the chemical detergents and sanitizers were used together, they removed the majority of organisms on every surface. A similar study conducted by Gibson et al. (2001) tested the effectiveness of cleaners used in the food industry against bacterial biofilms. The results showed that after just the cleaning program, there was still a significant amount of microorganisms on the surface. Therefore, the disinfectant stage was needed in order to eliminate any remaining microorganisms.

The results of both of these studies are comparable to the results of the current trial. The initial hypothesis of this trial was that the cleaning and sanitizing program would eliminate more of the bacteria on the associated cutting board surface than the sanitizing only program. The current trial used sanitizer infused wipes instead of a liquid sanitizer disinfectant used in previous studies. Sanitizer wipes are easy to use and can prevent cross-contamination if used on one surface and disposed of. However, some sanitizer wipes require a 4-minute minimum contact time and a rinse in order to work effectively (Bolton et al., 2013; Clorox, 2012). A study conducted by Bolten et al. (2013) looked at the efficiency of different sanitizer application methods against certain viruses on stainless steel surfaces. One of the methods was a sanitizer infused wipe, while the other was a sanitizer spray.

The study, found that the pre-moistened sanitizer wipes proved to be most effective in the removal of microorganisms, whereas the sanitizer spray caused cross-contamination between surfaces after spraying.

The results of the current study indicated that the sanitizing only program used on the associated cutting board on both turkey and salami type samples worked just as effectively as the cleaning and sanitizing program. Using a pre-moistened sanitizer wipe instead of the sanitizer spray could be one of the reasons as to why the levels are similar to that of the cleaning and sanitizing program. Using a sanitizer spray may have caused cross contamination between the other sample areas being tested. This trial was used as a means of determining which cleaning method is most effective in eliminating *L. innocua* and other microorganisms in a retail deli setting. The results concluded that both the cleaning and sanitizing method and the sanitizing only method are equally as effect in eliminating bacteria and other microorganisms from food contact surfaces thus resulting in a safer food-processing environment.

Trial 3

The objective of trial three was to determine if the purge inside the bags and/or the bag itself that contained the salami and turkey deli loaves tested positive for generic *Listeria* spp. (Table 3). Food Safety Inspection Service (FSIS) indicated that since 1990, the prevalence of *Listeria* has declined in federally inspected food processing facilities (USDA and FSIS, 2009). Out of 16 turkey samples (n = 16) and 16 salami samples (n = 16), there was zero positive generic *Listeria* spp. at the detection level capable of the 3M Environmental *Listeria* petrifilm.

In 2002, there were 54 cases of listeriosis between July and November. An investigation was completed to determine the source of the outbreak.

Table 3. Prevalence of generic *Listeria* spp. samples obtained from the purge of commercially processed deli loaves and plated on 3M Environmental *Listeria* petrifilm.

	Turkey (n = 16)	Salami (n = 16)
Percent Positive	0.00	0.00

FSIS found that the turkey deli meat from one specific plant was the cause of the outbreak. Knowing this, the FSIS developed new regulatory policies involving specific *L. monocytogenes* control programs. One year after implementing those policies, FSIS conducted a survey on the plants undergoing those policies. The FSIS reported a 25% decrease in *L. monocytogenes* positive samples detected by the regulatory testing program. Two years after the outbreak, FSIS reported a 40% decrease in human listeriosis cases. Ultimately, implementing these policies improved plant production and prevented cross-contamination of *L.monocytogenes* (Gottlieb et al., 2005).

The results from the current study support the notion that the implemented policies help to prevent the growth of *L. monocytogenes.* Pradhan et al. (2009) and Endrikat et al. (2010) conducted two similar risk assessments for *L. monocytogenes*. One dealt with contamination of *Listeria* in prepackaged verses retail sliced deli meat while the other dealt with listeriosis associated deaths due to contamination of *Listeria* from manufacture and retail. The results of both assessments indicated that *Listeria* contamination and illness is most likely to happen at the retail level because of time and temperature abuse. The current study contained turkey deli loaves unopened from a retail deli. Therefore, no time or temperature abuse could have occurred from the retail level resulting in zero positive *Listeria* spp. samples.

The initial hypothesis of this trial suggested there would be zero positive generic *Listeria* samples taken from the purge of the salami and turkey deli loaves. The results concluded that there were zero positive generic *Listeria* spp. samples. The FSIS policies have greatly improved the way food-processing facilities handle RTE meat and poultry. Those

policies along with antimicrobial agents added into the meat greatly reduce the chances of *Listeria* growth in processing facilities.

CONCLUSION

Microbial growth due to insufficient cleaning and sanitation practices may be a forceful issue in retail deli settings. The aim of this study was to evaluate temporary storage orientation of commensal bacteria and the impact of sanitizing programs on survivability of *Listeria innocua* on food contact surfaces in retail deli settings. Results obtained from trial 1 indicated that the face orientation of turkey had the lowest levels of microbial growth. This study provides additional data to suggest meat orientation placement is significant but dependent on type of deli meat in reducing microbial growth on food contact surfaces in retail deli settings. Results obtained from trial 2 indicate that both cleaning programs work equally as effective in removing *Listeria innocua* along with other types of microorganisms on food contact surfaces. This research, however, does not support the idea that cleaning and sanitizing is significantly more effective than sanitation only. Nonetheless, studies such as Gibson et al. (2001) and Somers and Wong (2004) agree that it is still highly recommend to use a cleaning and sanitizing method over the sanitation only method. In conclusion, this research determined the treatment orientation of RTE meat varies depending on meat product. Additionally, both cleaning programs were equally as effective in reducing cross-contamination and microbial growth on food contact surfaces in retail deli settings.

LITERATURE CITED

AOAC® Official Methods^{SM} (990.12 Aerobic Plate Count in Foods, Dry Rehydratable Film Methods).

Bolton, S.L., G. Kotwal, M.A. Harrison, S.E. Law, J.A. Harrison, and J.L. Cannon. 2013. Sanitizer efficacy against murine norovirus, a surrogate for human norovirus, on stainless steel surfaces when using three application methods. *Applied and Environmental Microbiology.* 79, 1368-1377.

Bourion, F., and O. Cerf. 1996. Disinfection efficacy against pureculture and mixed population biofilms of Listeria innocua and Pseudomonas aeruginosa on stainless steel, Teflon, and rubber. *Food Science*. 16:151-166.

Busta, F.F., T.V. Suslow, M.E. Parish, L.R. Beuchat, J.N. Farber, E.H. Garret, and L.J.

Carpentier, B. 1997. Sanitary quality of meat chopping board surfaces: a bibliographical study. *Food Microbiology*. 14, 31-37.

Center for Food Safety and Applied Nutrition and Food and Drug Administration. 2001. Guidelines for the microbiological examination of ready-to-eat foods.

Clorox. 2012. Clorox disinfecting wipes: usage instructions. Clorox, Oakland, CA. Accessed 21 June 2012.

Curiale, M.S., and C. Lewus. 1994. Detection of *Listeria monocytogenes* in samples containing *Listeria innocua*. *Journal of Food Protection*. 57, 1048-1051.

Dinev, T., G. Beev, M. Tzanova, S. Denev, D. Dermendzhieva, and A. Stoyanova. 2018. Antimicrobial Activity of *Lactobacillus plantarum* Against Pathogenic and Food Spoilage Microorganisms: A Review. *Bulgarian Journal of Veterinary Medicine.* 21, 253-268.

Donnelly, C.W. 2001. *Listeria monocytogenes*; a continuing challenge. *Nutr Rev*. 59, 183-194.

Duh, Y.H., and D.W. Schaffner. 1992. Modeling the effect of temperature on the growth rate and lag time of *Listeria innocua* and *Listeria monocytogenes*. *Journal of Food Protection*. 56, 205-210.

Dunsmore, D.G. 1981. Bacteriological control of food equipment surfaces by cleaning systems. I. Detergent effects. *J. Food Prot*. 44, 15-20.

Endrikat, S., D. Gallagher, R. Pouillot, H.H. Quesenberry, D. LaBarre, C.M. Schroeder, and J. Kause. 2010. A comparative risk assessment of *Listeria monocytogenes* in prepacked versus retail sliced deli meat. *Journal of Food Protection*. 4, 612-619.

European Food Safety Authority. 2007. Opinion of the scientific panel on biological hazards on microbiological criteria and targets based on risk analysis. *EFSA Journal*. 462, 1-29.

Farver, J.M., and P.I. Peterkin. 1991. *Listeria monocytogenes*, a foodborne pathogen. *Microbiol Rev*. 55, 476-511.

Fatima, M., M.M. Gil, T.R.S Brandao, P. Teixeira, and C.L.M. Silva. 2009. Sigmoidal thermal inactivation kinetics of *Listeria innocua* in broth: influence of strain and growth phase. *Food Control*. 20, 1151-1157.

Favaro, M., L. Sarmati, G. Sancesario, and C. Fontana. 2014. First case of *Listeria innocua* meningitis in a patient on steroids and etanercept. *JMM Case Reports*.

FDA. 2017. Food and Drug Administration Food Code. Chapter 4, 142-149.

Gandhi, M., and M.L. Chikindas. 2007. *Listeria*: a foodborne pathogen that knows how to survive. *Int. J. Food Microbiol*. 113, 1-15.

Gibson, H., J.H. Taylor, K.E. Hall, and J.T. Holah. 2001. Effectiveness of cleaning techniques used in the food industry in terms of the removal of bacterial biofilms. *Journal of Applied Microbiology.* 87, 41-48.

Gibson. K.E., P.G. Crandall, and S.C. Ricke. 2012. Removal and transfer of viruses on food contact surface by cleaning cloths. *Applied and Environmental Microbiology*. 78, 3037-3044.

Gottlieb, S.L., E.C. Newbern, P.M. Griffin, L.M. Graves, R.M. Hoekstra, N.L. Baker, S.B. Hunter, K.G. Holt, F. Ramsey, M. Head, P. Levine, G. Johnson, D. Schoonmaker-Bopp, V. Reddy, L. Kornstein, M. Gerwel, J. Nsubuga, L. Edwards, S. Stonecipher, S. Hurd, D. Austin, M.A. Jefferson, S.D. Young, K. Hise, E.D. Chernak, and J. Sobel. Listeriosis Outbreak Working Group. 2005. Multistate outbreak of listeriosis linked to turkey deli meat and subsequent changes in US regulatory policy. *Clinical Infectious Diseases.* 42, 29-36.

Gram, L., L. Ravn, M. Rasch, J.B. Bruhn, A.B. Christensen, and M. Givskov. 2002. Food Spoilage-interactions between food spoilage bacteria. *Int J Food Microbiol*. 78, 79-97.

James, C., B.A. Onarinde, and S.J. James. 2016. The Use and Performance of Household Refrigerators: A Review. Wiley online Library. V 16.

Joseph, B., S.K. Otta, and I. Karunasagar. 2001. Biofilm formation by *Salmonella* spp. on food contact surfaces and their sensitivity to sanitizers. *Int. Journal of Food Microbiology*. 64, 367-372.

Kodaka, H., S. Mizuochi, H. Teramura, and T. Nirazuka. 2005. Comparison of the compact dry TC method with the standard pour plate method (APAC official method 966.23) for determining aerobic colony counts in food samples: performance-tested method[sm]. *Journal of AOAC international.* 88, 1702-1713.

Krysinski, E.P., L.J. Brown, and T.J. Marchisello. 1991. Effect of cleaners and sanitizers on *Listeria monocytogenes* attached to product contact surfaces. *Journal of Food Protection.* 55, 246-251.

Kumar, Y., D.N. Yadav, T. Ahmad, and K. Narsaiah. 2015. Recent trends in the use of natural antioxidants for meat and meat products. *Institute of Food Technologists*. 14, 796-812.

Langsrud, S., and G. Sundheim. 1997. Factors contributing to the survival of poultry associated *Pseudomonas* spp. exposed to a quaternary ammonium compound. *Journal of Applied Microbiology*. 82, 705-712.

Leistner, L. 1994. Further developments in the utilization of hurdle technology for food preservation. *J. Food Engineering*. 22, 421-432.

Lianou, A., and J.N. Sofos. 2007. A review of the incidence and transmission of *Listeria monocytogenes* in ready-to-eat products in retail and food service environments. Journal of Food Protection. 70, 2172-2198.

Lin, C.M., K. Takeuchi, L. Zhang, C.B. Dohm, J.D. Meyer, P.A. Hall, and M.P. Doyle. 2006. Cross-contamination between processing equipment and deli meats by *Listeria monotygenes*. J Food Prot 69:71-79.

Loncarevic, S., T.W. Danielsson, and M.L. Tham. 1996. Prevalence of *Listeria monocytogenes* and other *Listeria* spp. in smoked and "gravid" fish. *Act Vet Scand*. 37: 13-8.

Marriot, N.G., M.W. Schilling, and R.B. Gravani. 2018. Principles of food sanitation. *Springer*. Sixth Edition.

Mbandi, E., and L.A. Shelef. 2001. Enhanced inhibition of *Listeria monocytgenes* and *Salmonella enteritidis* in meat by combinations of sodium lactate and diacetate. *Journal of Food Protection*. 64, 640-644.

Mertz, A.W., C.A. O'Bryan, P.G. Crandall, S.C. Ricke, and R. Morawicki. 2015. The elimination of *Listeria monocytogenes* attached to stainless steel or aluminum using multiple hurdles. *Journal of Food Science.* 80, 1557-1562.

Miller, A. J., T. Brown, and J. E. Call. 1996. Comparison of wooden and polyethylene cutting boards: potential for the attachment and removal of bacteria from ground beef. *Journal of Food Protection.* 59, 854-858.

Ndahetuye, J.B., O.K. Koo, C.A. O'Bryan, S.C. Ricke, and P.G. Crandall. 2012. Role of Lactic Acid Bacteria as a Biosanitizer to Prevent Attachment of *Listeria monocytogenes* F6900 on Deli Slicer Contact Surfaces. *Journal of Food Prot*. 75, 1429-1436.

Olsen, S., L. MacKinon,J. Goulding, N. Bean, and L. Slutsker. 2000. Surveillance for foodborne disease outbreaks-United States, 1993-1997. *Morb. Mortal. Wkly*. Rep. 49, 1-51.

Perez-Rodriguez, F., E.D. Van Asselt, R.M. Garcia-Gimeno, G. Zurera, and M.H. Zwietering. 2007. Extracting additional risk managers information from a risk assessment of *Listeria monocytogenes* in deli meats. *Journal of Food Protection*. 70, 1137-1152.

Pradhan, A.K., R. Ivanek, Y.T. Grohn, R.Bukowski, I. Geornaras, J.N. Sofos, and M. Wiedmann. 2009. Quantitative risk assessment of listeriosis associated deaths due to *Listeria monocytogenes* contamination of deli meats originating from manufacture and retail. *Journal of Food Protection*. 4, 620-630.

Rahman, A., and S. C. Kang. 2009. In Vitro control of food-borne and food spoilage bacteria by essential oil and ethanol extracts of *Lonicera japonica* Thunb. Food Chemistry. 116, 670-675.

Rajmohan, S., C.E.R. Dodd, and W.M. Waites. 2002. Enzymes from isolates of *Pseudomonas fluroescens* involved in food spoilage. *Journal of Applied Microbiology*. 93, 205-213.

Rod, S.K., F. Hansen, F. Leipold, and S. Knochel. 2012. Cold atmospheric pressure plasma treatment of ready-to-eat meat: Inactivation of *Listeria innocua* and changes in product quality. *Journal of Food Micobiology.* 30, 233-238.

Rodriguez, O., M.E. Castell-Perez, N. Ekpanyaskun, R.G. Moreira, A. Castillo. 2006 Surrogates for validation of electron beam irradiation of foods. *International journal of food microbiology.* 110, 117-122.

Sindelar, J.J., and A.L. Milkowski. 2011. Sodium Nitrite in processes meat and poultry meats: a review of curing and examining the risk/benefit of its use. American Meat Science Association. 3, 1-4.

Slade, P.J. 2003. Using surrogates in process validation studies. *Food Quality*. 33, 35-38.

Somers, E.B., and A.C.L. Wong. 2004. Efficacy of two cleaning and sanitizing combinations on *Listeria monocytogenes* biofilms formed at low temperature on a variety of materials in the presence of ready-to-eat meat residue. *Journal of Food Protection*. 67. 2218-2229.

Sperber, W.H., and M.P. Doyle. 2008. Compendium of the Microbiological Spoilage of Foods and Beverages.

U.S. Food Safety and Inspection Service. 2003. FSIS strengthens regulations to reduce *Listeria monocytogenes* in ready-to-eat meat and poultry products. Accessed 22 February 2005.

United States Department of Agriculture and Food Safety and Inspection Service. 2009. The FSIS microbiological testing program for ready-to-eat (RTE) meat and poultry products, 1990-2008.

United States Department of Agriculture and Food Safety and Inspection Service. 2012. Introduction to the microbiology of food processing.

United States Food and Drug Administration and Center for Food Safety and Applied Nutrition (FDA, CFSAN). 2001. Analysis and evaluation of preventative control measures for the control and reduction/elimination of microbial hazards on fresh and fresh-cut product. CFSAN, College park, MD.

Vorst, K.L., E.C.D. Todd, and E.T. Ryser. 2006a. Transfer of *Listeria monocytogenes* during mechanical slicing of turkey, bologna, and salami. *Journal of Food Protection*. 69, 619-626

Vorst, K.L., E.C.D. Todd, and E.T. Ryser. 2006b. Transfer of *Listeria monocytogenes* during slicing of turkey, bologna, and salami with stimulated kitchen knives. *Journal of Food Protection*. 69, 619-626.

Wagner, A.B. 2008. Food Technology and Processing. Introduction to the Microbiology of Food.

Yang, H., P.A. Kendall, L.C. Medeiros, and J.N. Sofos. 2008. Efficacy of sanitizing agents against *Listeria monocytogenes* biofilms on high-density polyethylene cutting board surfaces. *Journal of Food Protection*. 72, 990-998.

BIOGRAPHY

Jade Elaine Atkinson was born and raised in Harlingen, TX. She graduated high school in 2012. Upon graduation she attended Angelo State University. She was voted MFSA secretary in the Fall of 2016. She graduated with her undergraduate degree in Animal Science in the Fall of 2016. After completing her undergraduate degree, she chose to stay at Angelo State to complete her Masters of Science degree. She will complete her Masters of Science in the Fall of 2019.