THESIS

EFFICACY OF THE MICROTALLY® MITT FOR SAMPLING OF BEEF CARCASSES

Submitted by

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ABSTRACT

EFFICACY OF THE MICROTALLY® MITT FOR SAMPLING OF BEEF CARCASSES

Food safety is crucial in preventing foodborne illnesses and potential product loss due to pathogen contamination. Seven Shiga toxin-producing *Escherichia coli* (STEC) have been classified as adulterants in ground beef and any beef cuts intended for nonintact use. Therefore, testing carcasses and trimmings is a crucial step for identifying pathogenically contaminated products. Several sampling methods, including N60 excision, N60 Plus™, and continuous and manual sampling devices using the Microtally® Swab or Mitt, are widely used in industry for verification testing. Among these, the Microtally® Mitt (MT-Mitt) is a sterile cloth mitt approved for sampling beef trim. However, the efficacy of the MT-Mitt for sampling beef carcasses has not been validated. Therefore, the objectives of Phase 1 of this Study were to identify an appropriate lot size for carcass sampling (Study 1) and compare the MT-Mitt's effectiveness in bacterial recovery with that of carcass surface excision sampling (Study 2). The objective of Phase 2 was to compare the detection of pathogen index targets from carcass surface MT-Mitt samples with those from trim surface MT-Mitt samples from the same carcass.

For Study 1 of Phase 1, nine sampling lot sizes were evaluated, where each lot size was represented by the number of carcasses sampled using the same MT-Mitt. The lot sizes were 1, 2, 3, 4, 6, 8, 10, 12, and 14 carcasses, with each carcass comprising two sides. For example, a lot size of 1 was 1 carcass (2 sides), a lot size of 2 was 2 carcasses (4 sides), and so on, were sampled with the same MT-Mitt. Additionally, for all lot sizes, sampling was conducted at two

sites per carcass side using separate MT-Mitts: an upper sampling area that included the inside and outside round, and rectal area, and a lower sampling area that consisted of the chuck and brisket. Lot sizes 1, 2, 3, and 4 were replicated 5 times (n = 5) in plant A. Separately, lot sizes 4, 6, 8, 10, 12, and 14 were replicated 10 times (n = 10) in plant B. For Study 2 in Phase 1, carcass surface excision and MT-Mitt samples (n = 10) were obtained from two different plants on a different set of carcass sides than those utilized in Study 1. These samples were collected right after the harvest floor and in the chill cooler, and a lot size of 8 carcasses were used. The MT-Mitt samples were collected from the upper and lower areas of the sides, as previously described. Excision samples were collected using a sterile scalpel or a boning knife, depending on the facility, which was used to excise approximately 3 cm x 10 cm of carcass surface tissue from three locations in each sampling area (upper and lower). Excisions from the upper sampling area were from the hock, round, and rump, whereas excisions from the lower sampling area were from the foreshank, brisket, and short plate. All samples except for half of the samples collected in Study 2 were analyzed for indicator bacteria counts, using appropriate Petrifilm plates, to enumerate aerobic plate counts (APC), *Enterobacteriaceae* counts (EBC), total coliform counts (TCC), and *E. coli* counts (ECC). The remaining half of the Study 2 samples were analyzed for Shiga toxin-producing *Escherichia coli* (STEC) pathogen index targets. Data were analyzed using R using a linear regression model comparing lot sizes for Study 1, and excisions vs. MT-Mitt samples for Study 2.

Phase 2 was conducted over 13 consecutive production days in a commercial beef processing plant using the same upper and lower sampling areas as used in Phase 1 for sampling carcasses with the MT-Mitt. Beef trim samples were collected by swabbing either a box or combo bin with the MT-Mitt for 90 seconds. A lot size of 8 carcasses was used based on the

results from Phase 1, and a lot size of 5 boxes per pallet or a single trim combo bin was utilized for trim samples. Trim samples were correlated to carcass samples taken 48 hours previously. Both carcass and trim samples were evaluated for 8 pathogen index targets using polymerase chain reaction (PCR). One hundred and thirty carcass swab samples (a total of 1,040 carcasses) and 166 trim swab samples were collected and analyzed over the 13 days.

Regardless of lot size in Study 1, the mean APC of the upper and lower sampling areas ranged from 1.2 to 2.9 and 1.6 to 2.2 log CFU/sample at plant A, respectively, and 2.1 to 2.7 and 1.8 to 3.0 log CFU/g at plant B, respectively. For the upper sampling area, there was a difference ($P < 0.05$) between the APC of lot sizes 1, 2, and 3, at plant A, and lot sizes 8 and 10 at plant B, whereas for the lower sampling area, there was a difference ($P < 0.05$) between the APC of lot sizes 12 and 14 at plant B, but no difference between lot sizes 1-4 evaluated at plant A. Out of the 120 samples collected from all lot sizes and both sampling areas at plant B, 73%, 72%, and 3% had quantifiable (greater than the 0.7 log CFU/g detection limit) EBC (range: <0.7 to 4.3 log CFU/g), TCC (range: <0.7 to 4.1 log CFU/g), and ECC (range: <0.7 to 1.2 log CFU/g), respectively. In Study 2, the overall recovery of APC from MT-Mitt samples was greater ($P < 0.05$) than that obtained from the excision samples. Specifically, quantifiable APC (greater than the 1.1 log CFU/g detection limit) were recovered from 80% of the MT-Mitt samples collected from the upper sampling site and from 100% of the samples from the lower sampling site. In contrast, quantifiable APC (greater than the 0.5 log CFU/g detection limit) was recovered from 0% and 70% of the upper and lower sampling area excision samples, respectively. Overall, the mean APC of the MT-Mitt samples from the upper and lower sampling locations were <1.9 and 1.7 log CFU/g, respectively, and <0.5 and <0.9 log CFU/g, respectively, for the excision samples. For mitt and excision samples evaluated for pathogen index targets, there were no targets

identified for any samples. The results of the study indicated that a lot size of 8 carcasses (16 sides) was an appropriate practical sampling size for sampling of beef carcass surfaces using the MT-Mitt. Moreover, under the experimental conditions of the study, the MT-Mitt was more effective in recovering indicator organisms compared to the excision sampling method.

For Phase 2, across all 8 indicators (STEC, O157, O111, O103, O26, O145, O121, and O45) for all carcass samples collected in Round 1, only two samples had detectable values: one for STEC and the other for O45. Of the 166 beef trim samples collected, 76 total detectable values were identified across the 8 indicators. In Round 2, more similar results were seen between carcass and trim samples for index target prevalence, with an average of 4% difference between carcass and trim samples on days 1 and 2. Carcasses on day 3 outperformed trim samples by having a higher target prevalence. Overall, there were inconsistencies between the carcass and trim results, suggesting the need for additional testing to fully validate the MT-Mitt for carcass sampling.

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CHAPTER 1

REVIEW OF LITERATURE

Food safety is crucial in preventing foodborne illnesses and the potential loss of products due to pathogenic contamination. Consumers rely on beef processors to produce safe products that are free from pathogens, hence the importance of having a robust pathogen testing program. Pathogenic strains of *Escherichia coli* (*E. coli*) are of concern in the meat industry as they have been associated with numerous outbreaks over the years, particularly with non-intact beef products. The Jack in the Box *E. coli* outbreak in 1993 spurred critical advancements in food safety, such as Hazard Analysis Critical Control Point (HACCP) and the implementation and classification of seven Shiga toxin-producing *E. coli* as adulterants in ground beef (Murano et al., 2018; Weinroth et al., 2018). This literature review will provide an overview of pathogenic strains of *E. coli* and their significance in the beef industry, including interventions used to reduce the microbial load on carcasses during harvest, current sampling methods employed in the industry, regulatory testing requirements, and testing methods.

1.1 *Escherichia. coli (E. coli)*

E. coli is a gram-negative bacterium within the *Enterobacteriaceae* family, having both pathogenic and non-pathogenic serotypes. Pathogenic *E. coli* can be characterized into five groups: enteropathogenic, enterohemorrhagic, enteroinvasive, enterotoxigenic, and enteroaggregative. Out of these five groups, enterohemorrhagic *E. coli* strains are most pertinent to the meat industry due to the unique ability of these serotypes to produce Shiga toxins (Stx) that can cause severe illness (Batt, 2014; Fatima et al., 2025). The Stxs are the primary virulence factors associated with Shiga toxin-producing *E. coli* (STEC), with two main types being

prevalent, Stx_1 and Stx_2 (Strockbine et al., 1986; Gyles, 2007). Once STECs enter the body, the protein intimin, encoded by their *eae* gene, facilitates attachment of the STEC to the hosts intestinal epithelial cells, which helps progress disease symptoms (Garrido et al., 2006; Mora et al., 2007). Infection with STEC is associated with bloody diarrhea, which can lead to hemolytic-uremic syndrome (HUS) and hemorrhagic colitis (HC) in the most severe cases (Bosilevac and Koohmaraie, 2011; Bruyand et al., 2018).

There are many different strains of STEC, but *E. coli* O157:H7 is the most prevalent serotype in the meat industry, and it is most frequently isolated from individuals with foodborne illnesses. *E. coli* O157:H7 contains the O157 plasmid, which can replicate separately from chromosomal DNA, and can provide resistance to antibiotics as well as produce toxins, aiding in its pathogenicity (Lim et al., 2010). Additionally, *E. coli* O157:H7 is acid-resistant, allowing it to colonize and grow in the low-acidity environment of the stomach, and has an extremely low infectious dose, making this pathogen very virulent (Lim et al., 2010).

1.1.2 Relevance of Shiga Toxin-Producing E. coli to the Meat Industry

Cattle are known carriers and reservoirs of pathogenic *E. coli*, specifically the O157:H7 serotype, and shed them through fecal matter. They are typically asymptomatic carriers, making it challenging to identify affected cattle (Duffy et al., 2006). Feces contaminated with *E. coli* O157:H7 shed by the cattle can adhere to hides and then potentially contaminate carcasses during the harvest process. Beef trim is a common source of pathogenic *E. coli* and has been linked to numerous foodborne illness outbreaks, largely caused by undercooked, contaminated ground beef (Duffy et al., 2006).

Numerous outbreaks (primarily involving ground beef products) throughout history have led to regulatory reforms and large-scale food safety improvements, such as Hazard Analysis and

Critical Control Point (HACCP), and the classification of some pathogens as adulterants (Murano et al., 2018). The Jack in the Box outbreak in 1993 was a catalyst for these reforms, as undercooked hamburgers contaminated with *E. coli* O157:H7 were the cause of over 700 cases of foodborne illness and the death of four children (Seo et al., 2014; Murano et al., 2018; Weinroth et al., 2018). This led to HACCP being made a regulatory requirement in 1996, which placed an emphasis on preventative food safety measures as opposed to the reactive measures that had been utilized in the early 1990s and before. The new emphasis on preventive food safety measures also resulted in the establishment of the Food Safety Modernization Act (FSMA) in 2011 (Grover et al., 2016; Weinroth et al., 2018). The FSMA focuses on improving the food chain as a whole (not solely the meat industry) by implementing programs and rules such as requiring food traceability, introducing supplier verification programs, and establishing clear guidelines for proper transportation and pathogen mitigation in food products, to name a few (FDA, 2024). The emphasis placed on preventative measures also resulted in *E. coli* O157:H7 being classified as an adulterant in non-intact beef products (i.e., ground beef), along with six other non-O157:H7 STEC serogroups in 1994 and 2012, respectively (USDA, 2012). These serogroups were selected as adulterants because they have been prevalent throughout history in connection with illnesses and outbreaks linked to contaminated ground beef. According to Brooks et al. (2005), between 1983 and 2002, 71% of isolates recovered from foodborne illness cases were from six serogroups (O26, O45, O111, O103, O121, and O145). Furthermore, *E. coli* O157:H7 is responsible for an estimated 73,000 illnesses and 60 deaths every year in the United States (Mead et al., 1999).

1.2 Interventions Utilized to Reduce the Microbial Load on Beef Hides and Carcasses

Various interventions are used during the slaughter process to reduce the microbial load on beef hides and carcasses. Commonly utilized interventions include hide washing, trimming, water washes, and organic acid washes (Arthur et al., 2004).

1.2.1 Hide Interventions

Cattle hides can be contaminated with STEC primarily through fecal shedding, making them a major source of carcass contamination (Bosilevac et al., 2005; Koohmaraie et al., 2005). One study found that, on average, a majority of hides, at 4 plants, tested for *E. coli* O157:H7 and *Salmonella* were contaminated (46.9% and 89.6% respectively), while carcasses generally had less contamination (16.7% and 9.05% respectively) (Brichta-Harhay et al., 2008). When the hide is removed from the carcass, there is the possibility of fecal matter being transferred from the hide to the outer portions of the carcass. Research has demonstrated that hides with high levels of bacterial contamination are correlated with carcasses with high levels of bacterial contamination (Arthur et al., 2004). Sites that are typically associated with high levels of contamination are correlated with sites where initial cuts are made and where the hide comes into contact with the carcass when being removed (Bell, 1997). For example, the brisket and shank are considered high-risk areas as opening cuts, such as the stick wound and initial skinning cuts, are made in these areas, respectively (Reid et al., 2002; Antic et al., 2010). Reducing bacterial levels on beef hides is crucial in minimizing carcass contamination, and numerous interventions can be employed to achieve this.

Hide washing cabinets have been proven to be effective, easy to implement, and a cost-effective intervention in reducing bacterial load on beef hides. Cabinets are installed in line, allowing carcasses to enter, receive the intervention, and exit, all while remaining part of the production chain. Hide wash cabinets are typically located following exsanguination but before

initial cuts to the hide are made. (Bosilevac et al., 2005; Koohmaraie et al., 2005). These cabinets spray the hide with a diluted chemical spray. The chemicals used can vary, but sodium hydroxide at a 1.5% concentration has been widely researched and shown to be extremely effective in reducing generic *E. coli* as well as *E. coli* O157:H7 (Bosilevac et al., 2005; Koohmaraie et al., 2005). Sodium hydroxide is widely used not only because of its effectiveness in reducing bacterial load but also because it remains effective even when recirculated (Bosilevac et al., 2005). After receiving the sodium hydroxide wash, many carcasses receive a hide-on water wash, and the water can then be recycled and cleaned with a 1 ppm concentration of chlorine (Bosilevac et al., 2005). The water wash helps to increase the effectiveness of the sodium hydroxide (or other antimicrobial treatment), reduce employee exposure to highly concentrated levels of the base, and aid in removing loosened material from prior washes (Bosilevac et al., 2004; Bosilevac et al., 2005).

Vacuuming (with or without steam) the hide removal pattern lines aids in removing loose debris from the carcass and has been proven effective in reducing bacterial load and the amount of liquid present along the hide removal lines (Dorsa et al., 1996; Dorsa, 1997; Bosilevac et al., 2005). Visual reduction of fecal matter on the carcass is extremely important due to a zero-tolerance policy regarding fecal, milk, and ingesta on the outside of a beef carcass (FSIS, 2019). Steam vacuuming can be a viable and non-destructive alternative to visually trimming off contamination, which is discussed later on in this review. When a sodium hydroxide wash was paired with a water wash and vacuuming of pattern lines on carcass sides, it was found to reduce *E. coli* O157:H7 prevalence on hides from 44% before the wash to 16% after the wash (Bosilevac et al., 2005).

In addition to hide washing and steam vacuuming, chemical dehairing can also be used to minimize contamination between the hide and the carcass. Chemical dehairing is a multistep process that commonly begins with the application of a sodium sulfide solution, which removes hair and debris from the hide, followed by a hydrogen peroxide solution, which neutralizes the sodium sulfide, and then finally a water wash to remove the chemicals before dehiding (Bowling and Clayton, 1992; Nou et al., 2003). In a laboratory setting, chemical dehairing of pieces of hide was effective in reducing *E. coli* O157:H7 and aerobic plate counts (APC) from around 5 log CFU/cm^2 to less than 0.5 log CFU/cm^2 (Castillo et al., 1998a). However, mixed results have been reported when chemical dehairing is examined at the plant level. Schnell et al. (1995) found no significant difference in bacterial load between chemically dehaired hides and control cattle. The hides were visually cleaner, resulting in less need for trimming the visually contaminated outer portions of the carcass (Schnell et al., 1995). A later study with a larger sample size found chemical dehairing to be effective in reducing the presence of *E. coli* O157:H7 and overall bacterial load, especially when combined with subsequent interventions (Nou et al., 2003). Dehairing the hide can reduce its value, especially if it is destined for leather production, as the hide can be damaged during the chemical treatments (Antic et al., 2021). Furthermore, sodium sulfide is highly toxic and has negative impacts on the environment, and generates harmful waste (Dixit et al., 2015; Kanagaraj et al., 2015). Therefore, chemical dehairing has not been universally adopted and has been determined not to be practical for industry use.

1.2.2 Carcass Trimming

Once the hide is removed, various interventions are used to further reduce or eliminate the visual contamination and bacterial load on beef carcasses. A common method that can be employed is trimming the outer portions of the carcass that are visibly contaminated with feces,

milk, or ingesta using a knife (Hardin et al., 1995). As mentioned earlier, there is a zero-tolerance policy for these contaminants on beef carcasses. Trimming is effective in not only visually eliminating these contaminants but also aiding in reducing bacterial load on the carcass (Hardin et al., 1995; Prasai et al., 1995). Studies have explored the effect of trimming on the reduction of bacterial counts and found that trimming by itself significantly reduced bacterial populations by 1.7 logs on average, with one study finding a reduction as high as 3.0 logs (Prasai et al., 1995; Reagan et al., 1996).

Although trimming of beef carcass surfaces is effective in visually reducing contamination, visual cleanliness does not always correlate with microbiological cleanliness. (Prasai et al., 1995). Moreover, bacteria may not always be present in visible contamination and could be present elsewhere on the carcass. Additionally, trimming carcasses can generate a large amount of waste and can also aid in the spread of pathogens from the carcass to plant personnel's equipment, such as knives (Hardin et al., 1995). These downsides make extensive carcass trimming a less desirable option for plants, especially when considering the product loss. Many plants still utilize carcass trimming but focus on limiting product waste as much as possible, and avoid trimming large portions from the carcass.

1.2.3 Water Washes as a Carcass Intervention

Water washes after hide removal are an additional carcass intervention that can be utilized to reduce microbial load, and the use of hot water is considered to be one of the most effective interventions to reduce contamination (Bosilevac et al., 2006). It can be a viable alternative to trimming carcasses as it doesn’t result in product loss. The effectiveness of hot water vs. ambient water temperature has been researched, and hot water has been proven to be more effective in reducing microbial load compared to ambient water (Barkate et al., 1993;

Gorman et al., 1995; Dorsa et al., 1996). Hot water was found to reduce naturally occurring microbial populations on beef carcasses by 1.3 logs (Barkate et al., 1993), while in another study (Gorman et al., 1995), inoculated generic *E. coli* populations were reduced by 3.0 logs. It is important to note that hot water is most effective in reducing bacterial counts when the water is hot enough to raise the carcass surface to 74°C (165°F) for more than five seconds (Koohmaraie et al., 2005). The United States Department of Agriculture-Food Safety and Inspection Service (USDA-FSIS) recommends maintaining water temperatures between 74-85°C to achieve optimal bacterial reduction (Edwards and Fung, 2006).

When a hot water wash was compared to a lactic acid wash, the hot water wash reduced *E. coli* O157:H7 prevalence by 81% while the lactic acid wash reduced *E.coli* O157:H7 by only 35% (Koohmaraie et al., 2005). On the other hand, a study by Hardin et al. (1995) found hot water to be the least effective out of the interventions evaluated (trimming, hot water wash, or hot water wash followed by either a lactic acid or acetic acid spray), and that water washing had the highest spread of contaminants to other portions of the carcass. It is important to note that this study utilized a manual wash rather than an automated spray cabinet, which could account for the decrease in effectiveness.

Currently, hot water cabinets are widely used in the beef industry as an intervention. Typically, the carcass sides enter the cabinet, where rotating nozzles distribute the water to all parts of the carcass at a consistent temperature and pressure. The temperature, pressure, and distance from the nozzles to the carcass are critical in the effectiveness of the cabinet (Davey and Smith, 1989; Barkate et al., 1993). Castillo et al. (1998c) showed that using a wash cabinet where nozzles were 12.5 cm away from the carcass, spraying water at a temperature of 95°C and a pressure of 24 psi effectively reduced *E. coli* O157:H7 counts by approximately 3.7 logs. This

study inoculated carcass surfaces with feces inoculated with *E. coli* O157:H7, and investigated differences regarding pathogen reduction when the carcass was first inoculated and how soon it received the intervention (hot carcass wash). Carcasses were sprayed 5 minutes or 20-30 minutes after contamination with no difference in pathogen reduction between treatments.

1.2.4 Organic Acid Washes as a Carcass Intervention

Organic acid washes have been widely studied and have been shown to be an effective intervention for reducing microbial populations on beef carcasses after hide removal (Hardin et al., 1995; Dorsa, 1997; Castillo et al., 1998c). Among these, lactic acid is the most extensively researched and commonly used organic acid in the beef industry due to its proven ability to reduce bacterial levels (Koohmaraie et al., 2005). Research has shown that, compared to lactic acid concentrations of 0.75% and 2.5%, a 1.25% solution is the most feasible for industry use, as it does not cause detectable color or flavor changes in the meat industry while also reducing total aerobic plate counts (Woolthuis and Smulders, 1985). Additionally, a 4% lactic acid spray applied for 35 seconds was found to reduce coliforms to below detectable levels (Castillo et al., 2001). While there is variation in the concentrations used across studies, the USDA-FSIS currently limits lactic acid concentrations on carcass surfaces to a maximum of 5% with the majority of the industry using a 2% concentration on pre-evisceration carcasses (Bosilevac et al., 2006). Similar to water wash cabinets, lactic acid is administered to the carcass through an online cabinet where the lactic acid is warmed to approximately 42°C for maximum efficacy, as verified by Bosilevac et al., 2006. Additionally, lactic acid has demonstrated no effect on the organoleptic properties of the sprayed product, specifically smell and taste of both raw and cooked product (Cudjoe, 1988; Harris et al., 2006; Harris et al., 2012; Yu et al., 2024)

Although lactic acid effectively reduces microbial populations, using it at high concentrations could negatively impact the visual attributes of meat. Rodríguez-Melcón et al., (2017) utilized a 5% concentration of lactic acid on beef carcass surfaces and saw a low pH compared to controls, along with extremely low redness values (*a**). Using lactic acid in high concentrations can also have corrosive effects on plant equipment, highlighting the importance of using lactic acid at appropriate concentrations. Additionally, numerous studies have shown a reduced ability to lower microbial counts on carcasses when compared with a hot water wash, implying that the two interventions should be used in conjunction with each other (Koohmaraie et al., 2005; Bosilevac et al., 2006).

Aside from lactic acid, acetic acid has been investigated as an alternative organic acid for reducing bacterial contamination on beef carcasses. When acetic acid was applied to post-harvest beef carcasses, the acid spray significantly reduced aerobic plate counts, *E. coli* counts, and *coliform* counts (Van Ba et al., 2018). However, studies have shown differences in the antimicrobial effects of acetic acid compared to those of lactic acid. For example, Arthur et al., (2008) reported that 2% lactic acid reduced *E. coli* O157:H7, *Salmonella* Newport, and *Salmonella* Typhimurium by an average of 1.6 logs, while the average reduction obtained with the 2% acetic acid was 0.8 logs. It is worth noting that acetic acid has minimal impact on the organoleptic properties of the treated product. Studies have evaluated the sensory and color of treated beef cuts, beef trim, and ground product and found minimal negative effects on the flavor of cooked products when compared to control samples (Bell et al., 1986; Kotula and Thelappurate, 1994; Harris et al., 2012). In regard to color, acetic acid-treated samples had visually varying color compared to controls (distilled water). In some instances, treated samples were significantly lighter in appearance or exhibited slight two-toning; however, it was not

severe enough to affect consumers' choices when buying the product (Bell et al., 1986; Kotula and Thelappurate, 1994; Harris et al., 2012).

Other organic acids, such as formic, propionic, peroxyacetic (PAA), and citric acids, have also been investigated for their antibacterial properties. In one study comparing formic, propionic, lactic, and acetic acid at varying concentrations (1-2%), each organic acid effectively reduced *E. coli* O157:H7 and *Staphylococcus aureus* inoculated tissues, with formic acid providing the highest reduction (Raftari et al., 2009). Dixon et al., (1987) compared a mixture of citric acid and ascorbic acid against lactic acid and acetic acid, and found minimal differences between their effectiveness, but all of the acids were effective in reducing bacteria when compared to a control. Peroxyacetic acid is more routinely used in the poultry industry; however, some studies have explored its effectiveness in reducing microbial load on beef. When PAA was utilized during carcass chilling, it was ineffective in reducing *E. coli* O157:H7 and *Salmonella* Typhimurium at any concentration (up to 1000 ppm) and temperature (45 and 55°C) (King et al., 2005). Another study contradicted these results and found PAA to effectively reduce microbial counts during retail display of ground beef (Mohan et al., 2012). The use of citric acid and PAA had little to no effect on sensory and color attributes of the treated product, with PAA treated samples maintaining a red color longer than untreated samples (Dixon et al., 1987; Mohan et al., 2012). Formic acid has been found to turn meat brown immediately after application, and hence, it is not commonly used in the meat industry (Quartey-Papafio et al., 1980).

1.2.5 Multiple-hurdle Intervention Approach

Although the previously mentioned interventions are effective by themselves, a multiple-hurdle approach has been investigated and is often adopted by the meat industry as a more robust food safety option. Using multiple interventions throughout the harvest process has proven to

reduce microbial populations even more than when a sole intervention is used (Hardin et al., 1995; Castillo et al., 1998c; Bacon et al., 2000). For example, using steam vacuuming while the hide is on the carcass, then utilizing a hot water wash, followed by a lactic acid spray, and finally spot trimming if necessary, significantly reduces microbial populations and utilizes multiple interventions as safeguards for lingering bacteria (Dorsa et al., 1996; Arthur et al., 2004; Koohmaraie et al., 2005). Another study found that utilizing a multiple-hurdle intervention approach at four different plants resulted in contaminants being reduced by an average of 3.5 logs (Brichta-Harhay et al., 2008).

1.3 Regulatory Requirements and Sampling Methods used for Pathogen Detection

Product testing is a critical part of the beef supply chain, especially with the classification of seven STECs as adulterants in non-intact beef products (i.e., ground beef). The Food Safety and Inspection Service (FSIS) requires all federally inspected meat processing facilities to have a verified HACCP plan to improve food safety in their products. Relevant pathogens must be addressed in the HACCP plan if they are deemed a hazard reasonably likely to occur in that process. The seven STECs are considered as hazards reasonably likely to occur in almost all beef plants, hence why product testing is critically important. It is recommended by FSIS to test every production lot for STECs to ensure robust sampling, with each lot needing a negative test result before leaving the facility (FSIS, 2014). During high event periods (positive rate is 5% or greater within a set time), product sampling should be increased to primals, subprimals, and food-contact surfaces, as it can be indicative of a failure in a plant's process (FSIS, 2014).

Numerous interventions are implemented on the harvest floor to reduce pathogens on the carcass surface and to help reduce contamination entering the fabrication floor and final products. The microbiological testing of the final product is often used as verification that it is

free from pathogens of interest. Sampling and testing of the product are not a preventative measure, but rather a verification that the plant's processes, such as interventions and hygiene practices, are working appropriately.

Beef carcass testing for pathogenic *E. coli* is not commonly practiced in large plants in the United States; however, a carcass sampling program known as the Advanced Pathogen Testing and Carcass Certification Program, developed by IEH, has been granted a letter of no objection from FSIS. More specifically, the Advanced Pathogen Testing and Carcass Certification Program involves sampling carcasses at the end of the slaughter process or upon entry into the chiller (The American Meat Science Association, 2022). Sampling consists of lotting carcasses with no more than 10 carcasses per lot, followed by sampling using the N60 excision method, which is described later. Lots are required to be held until a negative confirmation is received, similar to trim testing. Although this program exists, there are currently no requirements for plants to test carcasses for pathogens. There is a zero-tolerance policy for visible fecal matter, milk, or ingesta on carcasses at the end of the slaughter process; however, pathogen control and testing are focused on the end product (USDA FSIS, 2020). Even with testing being focused on ground beef or trim product, numerous studies have identified that pathogens enter a plant on beef hides, which are then subsequently transferred to the carcass surface (Bell, 1997; McEvoy et al., 2000). These findings necessitate looking into carcass testing for pathogens as a potential option for improved food safety sampling.

Beef trim is most commonly tested for pathogen control verification in the US, despite our knowledge that contamination often occurs on the carcass. Therefore, samples are routinely taken from beef trim combo bins (~900 kg bin) to verify that the trim is free from pathogens, specifically the seven STECs classified as adulterants (Arthur and Wheeler, 2021). These combo

bins are categorized into lots, where test and hold procedures are then implemented. Test and hold procedures require the trim combos to be held before entering commerce until a negative test is obtained (Guerini et al., 2006; Arthur and Wheeler, 2021; Arthur et al., 2024). There are numerous sampling methods approved for use for pathogen detection in trim, including N60, N60 Plus, a manual sampling device (MSD), and a continuous sampling device (CSD).

1.3.1 N60 and N60 Plus Excision Methods

The N60 and N60 Plus excision methods are considered the "gold standard" in the meat industry for the detection of pathogens in final products, and the N60 Plus method has been issued a letter of no objection from FSIS (Wheeler and Arthur, 2018). The N60 excision method is the process of taking 60 slices from the surface of a carcass or from trim in a combo bin with a combined target weight of ~375 g (Wheeler and Arthur, 2018; Arthur and Wheeler, 2021). The N60 Plus method is similar; however, a fluted barrel is used by inserting it into 5 areas of the combo bin (four corners and center), and shaving trim surface material until the sampler is full (~165 g) (Wheeler and Arthur, 2018; Arthur and Wheeler, 2021). Improvements in sampling procedures from excision methods are critical, as the N60 and N60 Plus are labor-intensive, destructive sampling methods that take product away from commerce. Additionally, these methods sample a relatively small portion of the combo bin (~0.45 kg out of 900 kg) and might not be a robust form of sampling (Arthur and Wheeler, 2021). Even with these downsides, both of these methods are commonly compared when validating new sampling methods, such as the CSD and MSD (Wheeler and Arthur, 2018). Cloth-based sampling methods, such as the CSD and MSD, have now been mandated by USDA-FSIS to replace excision methods for sampling of domestic beef for FSIS inspection personnel; hence, there is a push for the exploration of these methods (FSIS, 2023; FSIS, 2024).

1.3.2 CSD and MSD Methods

In the past few years, researchers have been investigating sampling technologies that are non-destructive and sample a larger portion of the combo bin. Two sampling devices have been validated and approved for use on beef trimmings: the CSD and MSD. The MSD is a spun-bound olefin polymer sterile cloth that is rubbed across the lean surface of the combo bin to recover any pathogens present (Wheeler and Arthur, 2018). The cloth is vigorously rubbed across the surface of the combo bin for approximately 90 seconds, flipping the cloth over after 45 seconds (Beef Industry Food Safety Council, 2023). When compared to the N60 and N60 Plus methods, the MSD was found to recover organisms as efficiently as the excision methods when samples were both naturally contaminated or inoculated (Wheeler and Arthur, 2018; Arthur and Wheeler, 2021). In 2024, the Microtally® Mitt (MT-Mitt) was validated as being effective in recovering organisms when compared to excision methods (Arthur et al., 2024). The MT-Mitt utilizes the same cloth fabric as the MSD, but fits on the hand of the sampler for easier sampling.

The CSD is made from the same sterile spun polymer cloth as the MSD and MT-Mitt, but is attached to the bottom of the trim chute to sample trim prior to it entering the combo bin. The cloth is held in place by a stainless steel holder attached to the end of a trim conveyor belt, with the cloth and holder being replaced after each combo is filled (Wheeler and Arthur, 2018; Arthur and Wheeler, 2021). When the CSD was compared to N60 and N60 Plus excision methods, it was able to recover a similar amount of pathogen index targets, making it a viable option for future implementation (Wheeler and Arthur, 2018).

The proven efficacy of the CSD, MSD, and MT-Mitt enhances microbiological sampling techniques by offering a non-destructive, time-efficient method that reduces the risk of employee injury from scalpels and increases the sampling area compared to traditional excision methods

(Arthur et al., 2024). With these added benefits, many processing plants have adopted either a CSD, MSD, or MT-Mitt method.

When a lot tests positive for any of the seven STECs classified as adulterants, processors must follow their corrective actions outlined in their HACCP plan, which consists of an adequate lethality treatment to kill the pathogen (FSIS, 2020). Additionally, processors must maintain control of the product until it reaches another establishment, renderer, or landfill and receives documentation that it has been handled appropriately (FSIS, 2020).

1.4 Analysis of Microbiological Samples

Analysis of samples collected from beef trim is a critical part of sampling plans and allows plants the ability to verify numerous process controls, such as the effectiveness of cleaning procedures and process interventions. Indicator organisms, pathogen index targets, and polymerase chain reaction (PCR) are routinely used in the meat industry for quantification and classification of bacteria and/or pathogens.

1.4.1 Indicator Organisms in the Meat Industry

Indicator organisms are bacteria that can indicate the hygienic condition of an environment or food surface and can signify numerous things, such as the presence of a potential pathogen or a lapse in good sanitation procedures (Tortorello, 2003). Indicator organisms can include aerobic plate counts (APC), generic *E. coli*, and *Enterobacteriaceae,* which are all commonly tested for in the food industry. The APCs are routinely used as indicators of satisfactory hygiene and sanitation practices, with high log counts indicative of poor cleanliness in a facility (Tortorello, 2003; Kim and Yim, 2017). Generic *E. coli* and *Enterobacteriaceae* are organisms indicative of fecal and/or pathogenic contamination, with high counts associated with

significant fecal contamination (Barros et al., 2007). Detection of these organisms is most commonly done by plating small amounts of the sample on either Petrifilm or bacteriological agar plates and incubating at 35°C for a set number of hours (Tortorello, 2003). If samples indicate high log counts of generic *E. coli* and/or *Enterobacteriaceae,* further analysis should be conducted to determine the potential presence of pathogens.

1.4.2 Pathogen Index Targets

Pathogen index targets operate in a similar manner to indicator organisms, but are indicative of pathogenic bacteria without conclusively identifying pathogens (Arthur and Wheeler, 2021). Analyzing samples for pathogen index targets is beneficial because it does not trigger regulatory action, even when targets are detected. Commonly utilized targets in the meat industry are genes associated with Shiga toxin-producing *E. coli* or enterohemorrhagic *E. coli,* such as hemolysin (*hlyA*), intimin (*eae*), the fliCH7 gene, stx_1, stx_2, and O157 (Paton and Paton, 1998; Bosilevac and Koohmaraie, 2011; Arthur and Wheeler, 2021). These genes are indicative of pathogens being present, but can also be associated with non-pathogenic strains, resulting in confirmation tests being required in certain instances.

1.4.3 Polymerase Chain Reaction (PCR) and its Role in Pathogen Detection

Polymerase Chain Reaction (PCR) is a DNA-based identification method where an organism or a gene's DNA is amplified to increase the amount of DNA to a detectable amount (Schochetman et al., 1988). Polymerase chain reaction is an attractive method for detecting pathogens, as it is a quick and accurate way of detection compared to more conventional methods, such as plating (Panwar et al., 2023). Data gleaned from PCR assays is primarily used

in the meat industry as an alternative to traditional culture plating methods to test for the presence or absence of pathogens due to its speed and accuracy.

The PCR is performed through a series of cycles, with each cycle consisting of three steps. The first step consists of separating the duplicate strands of DNA that make up the alpha helix formation, which requires breaking the hydrogen bonds typically with high temperatures (95-100°C; Schochetman et al., 1988). After the strands have been separated, short strands of specific DNA sequences called primers, adhere to the corresponding DNA sequence from the first step (Schochetman et al., 1988; Dymond, 2013). Specificity of primers is key to a successful PCR reaction, as a low specificity primer can confound results by attaching to non-target sequences (Dymond, 2013). The last step is replication of the target sequence, where the targeted DNA doubles after each cycle (Schochetman et al., 1988). Twenty to forty reactions typically occur, which can result in upwards of 1,000,000 copies of the selected DNA sequence (Schochetman et al., 1988). In the meat industry, detection targets routinely include *stx*$_1$ and *stx*$_2$, *eae*, and gene sequences correlating to the seven O serogroups (Huszczynski et al., 2013).

There are numerous different types of PCR available, such as multiplex PCR, real-time PCR, species-specific PCR, and direct PCR, to name a few (Paton and Paton, 1998; Li et al., 2020). In the meat industry, multiplex PCR and real-time PCR are commonly used, as both of these methods are relatively quick and can detect multiple DNA sequences simultaneously (Paton and Paton, 1998; Aladhadh, 2023). Real-time PCR quantifies pathogen load by using probes and fluorescent dyes to detect pathogens of interest, as well as monitor the amplification of DNA in real-time (Mancini et al., 2016). Conventional PCR only quantifies DNA at the end of the PCR run, making the real-time assay a more attractive option for many companies.

Like any detection method, PCR has its drawbacks, particularly in sample preparation. Due to the power of PCR, contaminants in the prepared sample can be amplified, potentially leading to inaccurate results (Schochetman et al., 1988). This emphasizes the importance of maintaining good aseptic technique and a high degree of accuracy during the DNA extraction procedure. Additionally, primers must be extremely specific to the desired targets, with large differences to other targets (Yang et al., 2022). This issue primarily arises with multiplex PCR, however it should be considered as more targets are added to these assays.

Throughout this literature review, many sampling and testing procedures discussed have focused on the final product, such as trim. With current industry standards focusing on testing beef trim for pathogens, there is a necessity for test-and-hold procedures, thereby increasing the time it takes for a product to be released from the facility. Carcass sampling could prove to be a viable alternative, reducing the time the product is held in storage. The research undertaken in the following chapters examines a carcass sampling protocol for beef carcasses.

CHAPTER 2

DETERMINING LOT SIZE AND COMPARING MT-MITT AND EXCISION METHODS FOR BEEF CARCASS SAMPLING

2.1 Introduction

Beef is a major source of dietary protein in the United States. Ground beef is especially popular, accounting for 49% of retail sales in the US, with ground beef products worth $13 billion sold in 2023 alone, making it one of the most widely consumed beef products (National Cattlemen's Beef Association, 2024). Ground beef is a high-risk product for pathogens due to the mixing process during grinding, which allows potentially pathogenic bacteria on the carcass surface to be internalized, and the propensity of consumers to undercook ground beef. Therefore, it is crucial to ensure the safety of ground beef products; hence, numerous steps are taken to eliminate physical, chemical, and biological contaminants in beef processing plants. Specifically, food safety practices are employed to reduce and prevent the incidence of foodborne illnesses and potential product loss due to biological (pathogen) contamination.

Consumers are recommended to cook ground beef to 160°F to destroy any potential pathogens, but even with this recommendation, many undercook ground beef products, making food safety practices at the plant level crucial (FSIS, 2025). Moreover, previous studies have indicated that only 1 in 4 consumers use thermometers while cooking meat products (Adams, 2024). Each year in the United States, foodborne illnesses are estimated to affect about 9.9 million people. Of these cases, roughly 357,000 are associated with Shiga toxin-producing *E. coli* (STEC), a pathogen highly prevalent in ground beef (Scallan Walter et al., 2019). Additionally, from

outbreaks associated with Food Safety and Inspection Service-regulated products, reported from 2007-2012, 77% involved commercially sold raw meat, with 80% of those linked to beef (Robertson et al., 2016).

Most of the legislation and regulations surrounding non-intact beef products, such as ground beef, were spurred by the Jack in the Box *E. coli* O157:H7 outbreak in 1993, which resulted in over 700 illnesses across multiple states and the deaths of four children (Seo et al., 2014). The legislation included changes such as the classification of seven Shiga toxin-producing *E. coli* (STEC) as adulterants in non-intact beef products and implementation of Hazard Analysis Critical Control Point (HACCP; Murano et al., 2018; Weinroth et al., 2018). To control pathogens in the beef supply chain (specifically the STECs classified as adulterants) and ensure a safe product, routine testing is conducted in beef processing facilities.

Currently, pathogen testing is focused on the final product, primarily beef trim destined for ground beef, with numerous sampling methods approved for use. These methods include destructive sampling methods such as the N60 and N60 Plus excision methods, along with newer, non-destructive methods consisting of the continuous and manual sampling device (CSD and MSD, respectively; Wheeler and Arthur, 2018; Arthur and Wheeler, 2021). With many plants shifting to a non-destructive form of sampling, new technology such as the Microtally® Mitt (MT-Mitt) has emerged. The MT-Mitt is a sterile cloth mitt and has been issued a letter of no objection by the United States Department of Agriculture – Food Safety and Inspection Service for sampling beef trim. Although trim sampling is industry standard, it requires test-and-hold procedures to be implemented, as the product cannot leave the facility until a negative test result is achieved. This requires space to hold the product, and a delay in the time it takes for the product to reach consumers. Due to these impacts, carcass sampling is being investigated as an alternative to detect

pathogens earlier in processing and therefore intervene (e.g., organic acid wash, trimming, etc.) earlier. Thus, the objective of this research was to examine the efficacy of the MT-Mitt in efficiently sampling beef carcass surfaces for indicator bacteria before entering the hotbox cooler.

2.2 Materials and Methods

2.2.1 Sample Collection

The research conducted for this thesis consisted of two phases (Phase 1 and Phase 2). Phase 1, comprised of two parts and designated as Study 1 and Study 2, is described in this chapter (Chapter 2), and Phase 2 is described in Chapter 3. The objective of Study 1 in Phase 1 was to determine how many carcasses could be sampled with a single Microtally® Mitt (Fremonta Corporation, San Jose, CA) before it was no longer efficient in capturing bacteria. Nine sampling lot sizes (1, 2, 3, 4, 6, 8, 10, 12, or 14 carcasses) were identified prior to plant collection for sampling. For example, a lot size of 1 was 1 carcass (2 sides), a lot size of 2 was 2 carcasses (4 sides), and so on. Data for lot sizes 1, 2, 3, and 4 were collected in a one commercial beef processing plant (plant A), whereas lot sizes (4 [replicated again], 6, 8, 10, 12, and 14) were collected at another commercial beef processing plant (plant B).

Sampling was conducted using two MT- Mitts per lot size: one for sampling the upper half of the carcass sides and the other for the lower half. The upper sampling area consisted of the inside and outside round, and rectal area, and the lower sampling area consisted of the chuck and brisket. Each lot size evaluated at plant A was replicated 5 times (n = 5), resulting in 40 mitt swabs (4 lot sizes x 2 areas x 5 replicates). Lot sizes evaluated at plant B were replicated 10 times (n = 10), resulting in 120 mitt swabs (6 lot sizes x 2 areas x 10 replicates) at plant B, and a total of 160 mitt swabs collected between the two plants for Study 1. Each sample was obtained

by swabbing either the upper or lower area of the carcass for approximately 5 seconds with a force approximately equivalent to removing a spot of dried blood, for the entire lot size. For the first collection at plant A (lot sizes 1, 2, 3, and 4), swabbing occurred at the end of the harvest floor immediately following all harvest floor interventions. For the second collection at plant B, swabbing occurred again at the end of the harvest floor after the hot water cabinet, but before the lactic acid cabinet. The time between these two cabinets was 43.5 seconds. One of the individuals involved in the sample collection was responsible for identifying the carcasses for each lot size. This individual was positioned at the front of the sampling area at both plants and signaled when the sample collectors should begin and stop swabbing. This ensured that the same upper and lower areas on the same carcasses were swabbed and that the lot size was accurate. After swabs were taken, they were immediately placed in bags labeled with the relevant lot size, sampling area (i.e., upper or lower), and replicate number. The bags were then transported under refrigeration to the Eurofins Microbiological Laboratory (EML) in Omaha, NE, for microbiological testing. Samples collected at plant A were evaluated at the Eurofins lab on-site.

The objective of Study 2 of Phase 1 was to compare bacterial recovery from MT-Mitt swabs and carcass surface excision samples. Two iterations of Study 2 were performed at two different commercial beef processing plants. For both iterations, samples were collected from carcasses that were different from those utilized in Study 1. For the first iteration, excision and swab samples were collected in the hotbox cooler, using a carcass lot size of 8 with 10 replicates (n = 10). Swabs were collected from the leading side of each carcass, and excisions were taken from the trailing side. The decision to use one lot size was made due to the time constraints associated with the excision of samples. Carcasses were railed off in the hotbox cooler to be able to obtain excisions and swab samples. The swabbing technique was the same as in Study 1, with

separate MT-Mitts used to swab the upper and lower areas of the sides. For excisions, one sterile scalpel (Swann-Morton, Sheffield, England) per replicate was used to excise samples (approximately 3 cm x 10 cm) from 3 spots in each sampling area (upper and lower). Excisions for the upper sampling area were always from the hock, round, and rump. For the lower excision area, 3 cm x 10 cm areas were removed from the foreshank, brisket, and short plate. Excisions and swabs from the upper and lower sampling areas were placed in separate bags and transported to the EML in Omaha, NE, under refrigeration, along with the MT-Mitt samples collected from plant B in Study 1.

The second iteration of Study 2 was conducted at a different commercial beef plant, with hot carcasses on the harvest floor and cold carcasses in the sales cooler. A lot size of eight was replicated 10 times (n = 10) on hot carcasses exiting the harvest floor, and again on different cold carcasses in the sales cooler. Excision samples were collected by plant employees using a boning knife. Upper excision samples were collected from the round and mid-loin, and lower excision samples were collected from the chuck. All samples were collected while the carcasses were moving on the rail rather than being railed off, as the carcasses in the previous iteration. The swabbing technique stayed the same as in Study 1 and the previously mentioned excision iteration. Within the first 5 replicates for hot and cold carcasses, the leading side was swabbed, and the trailing side was excised. For the next 5 replicates, the leading side was excised, and the trailing side was swabbed. Within each eight-head lot, mitts were flipped over after the fourth carcass to ensure equal bacterial recovery, and knives used for excision were cleaned with alcohol after every fourth carcass. It is important to note that four carcasses were always collected consecutively, but to ensure proper sanitization for the next four, a break was sometimes taken between the first and second set of four within the eight-head lot. Swabs from

the upper and lower sampling areas were immediately placed in bags, separated by repetition, and upper and lower excision samples were immediately placed in the same bag, divided by repetition. All samples were taken to the Eurofins lab in the plant for microbiological testing.

2.2.2 Microbiological Analysis

Samples for lot sizes collected at plant A (1, 2, 3, and 4), and hot and cold excision and swab samples from the second iteration of Study 2 were evaluated for Aerobic Plate Counts (APC) or Shiga toxin-producing *Escherichia coli* pathogen index targets, respectively. The samples from plant B in Study 1 and the samples from the first iteration of Study 2 were analyzed for APC, *Enterobacteriaceae* counts (EBC), total coliform counts (TCC), and *Escherichia coli* counts (ECC) testing. Mitts analyzed for APC, EBC, TCC, and ECC were enriched with approximately 200 mL of tryptic soy broth (BAC*Gro*™, Warminster, PA). Excision samples collected from the first iteration of Study 2 were enriched with tryptic soy broth at a ratio of 1: 2 (sample weight: broth weight).

Prepared samples were mechanically pummeled (Interscience BagMixer 400) for 1 minute and then serially diluted 10-fold. Appropriate dilutions were plated on Petrifilm Aerobic Count Plates, *Enterobacteriaceae* Count Plates, Total Coliform Count Plates, and *Escherichia coli* Count Plates (3M, St. Paul, MN). The plates were incubated at 35 ±1°C for 48 hours. Colony counts were then converted to CFU/g (samples collected at plant B in Study 1 and the first iteration of Study 2) or CFU/sample (samples collected at plant A in Study 1).

Excision and mitt samples collected in the second half of Study 2 were evaluated for pathogen index targets, using a real-time polymerase chain reaction (PCR) detection system. Upon arrival at the laboratory, approximately 200 mL of Modified Tryptic Soy Broth (Gold Standard Diagnostics, Horsham, PA) was added to each MT-Mitt and excision sample, followed

by mechanical pummeling (Interscience BagMixer 400, Paris, France) for 1 minute. Samples were then incubated at 41.5°C for a minimum of 10 hours. An aliquot from the upper and lower mitts of each replicate was combined into a 5mL tube for PCR analysis. An aliquot from the excision samples of each replicate was also placed in a 5mL tube for analysis. From the tube, a 30 µL aliquot of the enrichment was added to 70 µL of pre-weighed lysis buffer, consisting of Lysis Buffer S (a proprietary buffer) and proteinase K, to generate the lysate. The lysate was heated on heating blocks (Thermo Fisher Scientific, Waltham, MA) to 37°C for 20 minutes, followed by 95°C for 10 minutes, and then allowed to cool for 5 minutes at 4°C. The lysate was then kept refrigerated (4°C) until the PCR assay.

A commercially available BAC*Gene* real-time PCR kit, BAC*Gene* Mplex STEC Screen (Gold Standard Diagnostics, Horsham, PA) was used. The kit screened samples for the *stx*$_1$, *stx*$_2$, and *eae* genes. The previously prepared lysate, and the Master mix from the prepared kit, were centrifuged for 30 seconds before 5 µL of lysate was added to 20 µL of Master Mix (Fisher Scientific, Waltham, MA). Negative and positive controls, consisting of ultra-pure distilled water and plasmid from the PCR kit, were utilized for every run. After the Master Mix and lysate were mixed, tubes were vortexed for 15 seconds at 1150 rpm (Fisherbrand™ Scientific Analog vortex) and then centrifuged for 30 seconds using a Fisherbrand™ Microplate Centrifuge (Fisher Scientific, Waltham, MA). Samples were then run in a Bio-Rad CFX96 Deep Well™ Real-Time PCR machine (Bio-Rad Laboratories, Inc., Hercules, CA) or an AriaMX Real-Time PCR machine (Agilent Technologies, Inc., Santa Clara, CA). Data were analyzed using PURE software version 1.03 for the STEC (Gold Standard Diagnostics, Horsham, PA). Samples that had a value for either *stx*$_1$ or *stx*$_2$ AND the *eae* gene were presumed positive for STEC.

2.2.3 Statistical Analysis

Statistical analysis of the *Escherichia coli* counts (ECC), total coliform counts (TCC), and *Enterobacteriaceae* counts (EBC) was not performed as a large number of the samples had non-detectable plate counts. Statistical analysis of STEC signal data collected in the second iteration of Study 2 was also not performed, as no signals were detected across any mitt or excision sample. The APC data were analyzed in R Studio version 2023-04-05 using the tidyverse (Wickham et al., 2019), emmeans (Lenth, 2025), multcomp (Hothorn et al., 2008), and dplyr (Wickham et al., 2025) packages. All tests were conducted at the $\alpha = 0.05$ level. A linear regression model was fit with APC as the response variable for both studies. For Study 1, a fixed effect was included for lot size, and swabbing zone (upper or lower) was treated as a blocked factor. Lot size data from each plant was analyzed separately. The mean APC for each lot size was compared for upper and lower sampling areas using emmeans. Assumptions of constant variance and normal residuals were assessed using residual diagnostic plots. For the first iteration of study 2, swabbing zone (upper or lower) and type (MT-Mitt or excision) were treated as blocking factors. The mean APC for each sampling method was compared between the upper and lower sampling areas using emmeans. Assumptions of constant variance and normal residuals were again assessed using residual diagnostic plots.

2.3 Results

The mean (log CFU/sample or log CFU/g ± standard deviation [SD]) APC for Study 1 comparing bacterial recovery from the swabs from the upper and lower carcass sampling areas in relation to lot size is shown in Figures 2.1 and 2.2. At plant A, regardless of lot size, APCs of the upper and lower swab areas ranged from 1.2 to 2.9 and 1.6 to 2.3 log CFU/sample, respectively. There was a statistically significant difference ($P < 0.05$) among the upper lot sizes of 1, 2, and 3, with lot size 2 having the highest CFU/sample recovery. Among the lower lot sizes, there was no

statistical difference ($P < 0.05$) between any of the lot sizes. Microbial analysis for *Escherichia coli* counts (ECC), total coliform counts (TCC), and *Enterobacteriaceae* counts (EBC) were not performed due to constraints within the plant lab. For plant B, regardless of lot size, APCs of the upper and lower swab areas ranged from 2.1 to 2.7 and 1.8 to 3.0 log CFU/g, respectively. For the upper swabs, there was a statistical difference ($P < 0.05$) between APCs of lot sizes 8 and 10, whereas for the lower swab samples, there was a statistical difference ($P < 0.05$) between APCs of lot sizes 12 and 14. As previously mentioned, the ECC, TCC, and EBC data were not statistically analyzed as the majority of samples had non-detectable plate counts (detection limit: 0.7 log CFU/g). Specifically, out of the 120 samples collected at plant B in Study 1, 97% had ECC below the bacterial analysis detection limit. For the remaining 3% of samples, ECC ranged from <0.7 to 1.2 log CFU/g. For TCC and EBC, 28% and 27% of the samples, respectively, had non-detectable plate counts. For samples from which TCC and EBC were recovered, levels ranged from <0.7 to 4.1 log CFU/g and <0.7 to 4.3 log CFU/g, respectively.

Mean (log CFU/g ± SD) APC recovered from carcass swabs and excised carcass surface samples collected from the first iteration of Study 2, with a lot size of 8, are presented in Table 2.1. For the carcass swabs, quantifiable APC were recovered from 80% of the MT-Mitt samples collected from the upper sampling site and from 100% of the samples collected from the lower sampling site. For the excised samples, aerobic bacteria were recovered from 0% and 70% of the upper and lower sampling area excision samples, respectively. Aerobic plate counts for both swabs and excisions ranged from 0.5 to 3.0 log CFU/g. The APC recovered from the swabs and excisions were different ($P < 0.05$), with the swabs having a greater bacterial recovery for both upper and lower sampling sites. It should be noted that the detection limit of the microbial analysis was different for the swabs and excisions, with a detection limit of 1.1 log CFU/g for

swab samples and a detection limit of 0.5 log CFU/g for excision samples. The difference in detection limits is attributed to differences in the amount of diluent added to the two sample types. For the swabs, 200 mL of diluent was added to all samples, whereas for the excised carcass surface samples, a 1:2 ratio of sample weight to the volume of diluent was added. Statistical analyses were again not conducted for ECC, TCC, and EBC from the first iteration of Study 2, as most of the excision samples had non-detectable plate counts. The percentage of samples below the detection limit was 95% for TCC, 100% for ECC, and 95% for EBC. For samples from which TCC and EBC were recovered, counts were 0.5 log CFU/g.

Samples collected during the second iteration of Study 2 yielded no STEC signals. Three genes (*stx*$_1$, *stx*$_2$, and *eae*) were utilized for STEC detection, with a presumptive positive identified as the detection of a *stx*$_1$ or *stx*$_2$ gene AND the *eae* gene. Not only were there no presumptive positives, but no individual gene signals were detected for any mitt or excision samples.

2.4 Discussion

The MT-Mitt has been established as a viable option for pathogen testing on beef trim, but using the MT-Mitt for carcass sampling requires determining the number of carcasses that can be swabbed before the MT-Mitt needs to be changed to ensure efficient bacterial recovery (Arthur et al., 2024). The mean APC of swab samples from the upper sampling area in Study 1 (Figures 2.1 and 2.2) suggested that swabbing should be performed for a lot size of 8, as there were differences in bacterial recovery between 8 and 10 carcasses. On the other hand, results for the lower sampling area swab samples suggested that up to 12 carcasses could be sampled as the APC significantly (P<0.05) decreased only when the number of carcasses increased to 14. Between the upper sampling area lot size of 8 and the lower sampling area lot size of 12, there

were minimal differences in bacterial recovery (2.7 vs. 3.0 log CFU/g, respectively). Additionally, a lot size of 8 results in a greater number of samples tested, and fewer carcasses that will need additional interventions if the lot tests positive. Although a lot size of 1, 2, and 3 heads were evaluated, from an operational standpoint, changing mitts that frequently would be inefficient and time-consuming compared to an 8-head lot. Based on these reasons, a lot size of 8 carcasses was selected as the ideal lot size for MT-Mitt sampling of carcasses moving forward.

A previous study that also sampled carcasses using two sterile gauze swabs (one for the upper sampling area and one for the lower sampling area) used a lot size of 12 carcasses for analysis of APCs, TCCs, and ECCs (Van Donkersgoed et al., 1997). Unlike the current study, these researchers were not sampling every carcass, but their decision was primarily based on line speed and the ability of workers to collect samples rather than bacterial recovery. Jericho et al. (1994) used a lot size of 5 carcasses for sample collection; however, they were also limited by plant design and the ability of workers to sample. Studies can utilize different lot sizes but should follow the Beef Industry Food Safety Council's lotting guidelines for pathogen testing, which requires that justification be provided when a lot size greater than one carcass is used for testing purposes, as was provided through Study 1 (Beef Industry Food Safety Council, 2020).

Limited research has been conducted on the efficacy of the MT-Mitt for sampling beef carcasses, but numerous studies have investigated the use of the MT-Mitt or swab-based methods on beef trim. Studies have compared the continuous sampling device (CSD) and the manual sampling device (MSD), both of which use a sampling cloth, to the N60 and N60 Plus excision methods (Wheeler and Arthur, 2018; Arthur and Wheeler, 2021). All four sampling methods were evaluated against each other on beef trim with different lean points, in various configurations that are routinely used in a commercial beef plant. It was determined that

excision-based methods were more effective at detecting total bacterial counts, whereas swab-based methods were more effective at detecting pathogen index targets (Wheeler and Arthur, 2018; Arthur and Wheeler, 2021). Overall, the swab-based methods were found to be as effective, if not more effective in certain situations, than the excision methods, resulting in a letter of no objection from FSIS for use of the swabs on beef trim (Wheeler and Arthur, 2018; Arthur and Wheeler, 2021). The MT-Mitt was designed to improve the ease of use for sampling personnel, as it can be fitted over the hand rather than a piece of cloth, which can be harder to maneuver. Arthur et al. (2024) performed a study investigating the efficacy of the MT-Mitt compared to the MSD, N60, and N60 Plus excision methods. The MT-Mitt was found to be equivalent in effectiveness to the MSD and excision methods (Arthur et al., 2024).

In the first iteration of Study 2, between excisions and swabs, swabs had greater bacterial recovery than excision sampling. Testing beef trim by excision, also referred to as N60 or N60 Plus excision sampling, is a common practice in the industry (Wheeler and Arthur, 2018). The N60 excision sampling is known as the gold standard for trim testing, and it is essential that new methods, such as swabs, can detect equal or more pathogens than excision sampling. The N60 excision sampling for beef trim consists of taking ~approximately 60 surface excisions from a minimum of one combo bin to a maximum of five combo bins, resulting in a ~approximately 375g sample (Beef Industry Food Safety Council, 2023). The same principle applies to carcass sampling, with excisions taken totaling roughly 375g from at least one carcass (Beef Industry Food Safety Council, 2023). The N60 Plus sampling utilizes the same lotting scheme; however, a coring device is used in five different spots (four corners and the middle) on the combo bin to obtain product samples (Beef Industry Food Safety Council, 2023). Results from Study 2 support that swabs can pick up more bacteria than excision samples, most likely due to the larger surface

area captured by swabs. Similar results were found when continuous and manual sampling devices were compared to the N60 Excision sampling methods (Wheeler and Arthur, 2018; Arthur and Wheeler, 2021; Arthur et al., 2024). Wheeler and Arthur (2018) determined that swab samples recovered an equal or slightly greater amount of bacteria, whereas our results indicated that swabs picked up a significantly greater amount of bacteria. Similar results were observed in a later study where there was no significant difference between MT-Mitt and excision samples for indicator counts (Arthur et al., 2024). Results from the second half of Study 2 aligned more closely with these results, as there was no difference between swab and excision samples for STEC indicators. These differences could be attributed to the use of a surrogate inoculum in the 2018 study and differences in plant practices, such as interventions used, layout of the plant, etc. Additionally, in previously performed swab vs. excision studies, pathogen index targets were tested using polymerase chain reaction (PCR) technology to determine the presence of pathogens along with indicator counts; however, Study 1 and the first iteration of Study 2 only analyzed samples for indicator counts.

2.5 Conclusions

The results from the current studies suggest that the MT-Mitt is a promising and effective method for sampling beef carcass surfaces. The MT-Mitt demonstrated greater efficiency in recovering indicator organisms compared to the traditional N60 excision method. Based on these findings, a lot size of eight carcasses is recommended for future studies, as it was determined to be the most appropriate lot size among the nine evaluated. As these were preliminary investigations, additional research is warranted to compare the performance of the MT-Mitt on trim versus carcasses to fully validate its application for carcass sampling.

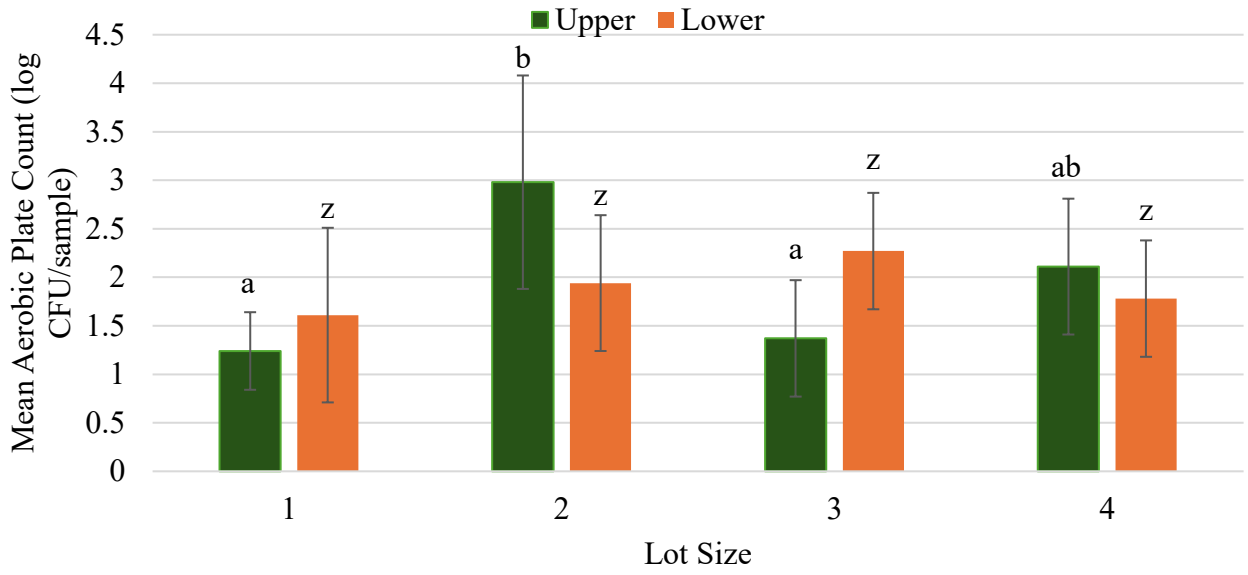

Figure 2.1. Mean (log CFU/sample ± standard deviation; n = 5) aerobic plate counts recovered from the upper and lower carcass sampling areas evaluated at plant A in Study 1. MT-Mitt swabs were taken after all harvest floor interventions before entering the hot box cooler.

[a-b]across bars (representing the upper sampling area), marginal means without a common superscript letter are different ($P < 0.05$).

[z]across bars (representing the lower sampling area), marginal means without a common superscript letter are different ($P < 0.05$).

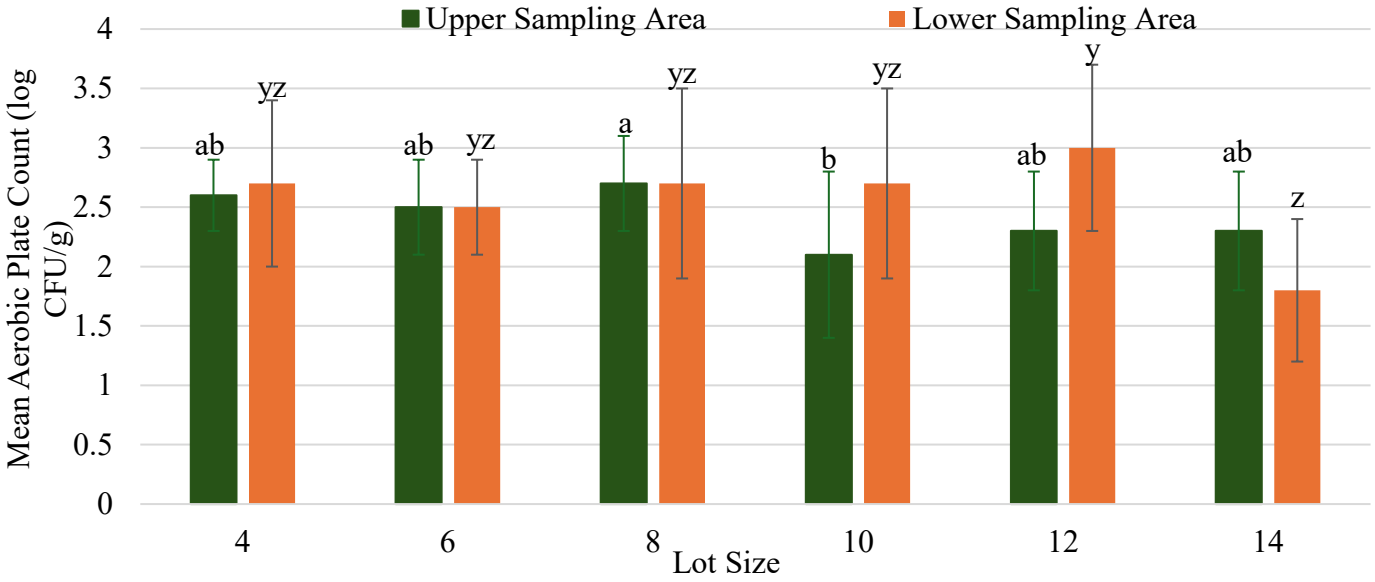

Figure 2.2. Mean (log CFU/g ± standard deviation; n = 10) aerobic plate counts recovered from the upper and lower carcass sampling areas evaluated at plant B in Study 1. MT-Mitt swabs were taken after the hot water wash and before the lactic acid spray.

[a-b]across bars (representing the upper sampling area), marginal means without a common superscript letter are different ($P < 0.05$).

[y-z]across bars (representing the lower sampling area), marginal means without a common superscript letter are different ($P < 0.05$).

Table 2.1. Mean (log CFU/g ± standard deviation; n = 10) aerobic plate counts (APC), recovered from the upper and lower carcass sampling areas for MT-Mitt swabs and excisions in the first iteration of Study 2. Swabs and excisions were taken in the hotbox cooler after a hot water wash and lactic acid spray.

Sampling Method	**APC**	
	Upper Sampling Area	Lower Sampling Area
MT-Mitt swabs	**<1.9±0.8[a,1]**	**1.7±0.3[a]**
Excisions	<0.4±0.0[b,2]	<0.9±0.8[b,3]

[a-b]Within each column (representing upper or lower sampling area), marginal means without a common superscript letter are different (P<0.05).
< indicates one or more of the samples had plate counts below the bacterial detection limit (1.1 log CFU/g for swab samples or 0.5 log CFU/g for excision samples).
[1] 20% of samples had counts below the bacterial analysis detection limit.
[2] 100% of samples had counts below the bacterial analysis detection limit.
[3] 30% of samples had counts below the bacterial analysis detection limit.

CHAPTER 3

EVALUATING THE EFFICACY OF THE MT-MITT SAMPLING METHOD ON BEEF CARCASSES COMPARED TO BEEF TRIM

3.1 Introduction

Ground beef is one of the most highly consumed beef products in the US, with it accounting for 49% of retail sales and $13 billion sold in 2023 alone (Mosier, 2024). When beef is ground, it increases the chance of any potential pathogens present on the surface being internalized, which in turn increases the food safety risk, especially if the product is undercooked. Hence, the beef industry has invested considerable effort to ensure that the products consumers receive are safe for consumption.

One of the primary steps in this is testing the products for the presence of pathogens. For example, trimmings and the finished ground beef products are routinely tested for microbiological quality, and to verify that the product is free of contamination with *E. coli* O157:H7 and six non-O157 Shiga toxin-producing *E. coli* serogroups, which are considered adulterants in nonintact beef products (USDA, 2012). The product cannot be released into commerce until a negative test result is achieved, necessitating the use of test-and-hold procedures. This takes up space in the plant to hold the product, as well as a delay in the time it takes for the product to enter commerce, potentially having a negative impact on its shelf life.

It is widely known that pathogenic contamination enters a beef processing facility with cattle through the presence of feces on cattle hides, which can then be transferred to the surface of the carcass. Even with this knowledge, testing of carcasses for pathogens is not widely used in

industry, as emphasis is placed on product testing (i.e., beef trim). Testing carcasses may aid in the early detection of pathogens, reduce the space required to hold products until a negative test result is received, and shorten the time it takes for products to enter commerce.

Phase 1 (Chapter 2) of this research identified that the MT-Mitt was more effective in recovering bacteria on carcasses when compared to N60 excision methods (industry gold standard), and that a lot size of eight carcasses (16 sides) should be used going forward. Based on these results, the objective of Phase 2 was to determine the efficacy of the MT-Mitt in recovering potential pathogen index targets, corresponding specifically to the seven Shiga toxin-producing *E. coli* serogroups classified as adulterants, from beef carcasses. Additionally, the pathogen index target recovery results from sampling of carcass lots were correlated with the pathogen index target recovery results obtained from MT-Mitt sampling of corresponding beef trim.

3.2 Materials and Methods

3.2.1 Sample Collection

Sample collection was performed in a commercial beef processing plant during its regular production hours. MT-Mitt swab sampling of carcasses was performed prior to entering the hot box for chilling, and the trim that was swabbed corresponded to carcasses that were swabbed 48 hours prior. A lot size of eight carcasses (16 sides) was utilized based on results from Phase 1 (Chapter 2) for Phase 2. In this phase, all trim from tested carcasses went into fully cooked products; therefore, pathogen index targets were used, as positive trim lots received a lethality treatment (cooking step).

Sample collection was conducted in two rounds: one (round 1) when the plant initially opened, and a second (round 2) a few months later, once the plant was processing more animals.

The carcass and trim samples were collected over 13 days in round 1 and over 3 days in round 2. Sampling of carcasses took place at the end of the harvest floor, after the harvest floor interventions (hot water wash, lactic acid spray, and hot fat trimming) but before carcasses entered the hotbox cooler. The carcasses were sampled using the MT-Mitt, with separate MT-Mitts used to sample the upper and lower halves of all eight beef carcasses within each lot. The upper sampling area included the inside and outside round, rectal area, hock, and down the midline, while the lower sampling area consisted of the chuck, brisket, neck stick wound, and up the midline (Chapter 2). The plant processed approximately 80 animals per day in round 1, resulting in 10 lots of carcasses sampled per day (n = 10) for 13 days, and a total of 260 carcass MT-Mitt samples (10 samples x 2 sampling areas x 13 days). During round 2 of data collection, the plant processed approximately 800 animals per day, resulting in a total of 2,048 carcasses sampled, comprising 512 MT-Mitt samples (i.e., 256 mitts for the upper sampling area and 256 mitts for the lower sampling area), again with eight carcasses per lot. Each sample was obtained by swabbing either the upper or lower area of the carcass for approximately 5 seconds with a force approximately equivalent to removing dried blood. For round 1, MT-Mitt samples collected from the upper and lower sampling areas were placed in separate sterile bags and transported under refrigeration overnight to the EML, Omaha, NE, for microbiological testing. Samples collected in round 2 were processed in the Eurofins laboratory located in the plant.

For both rounds, sampling of trim was conducted by swabbing the surface of the product in a box or combo bin using the MT-Mitt, with a force equivalent to scrubbing dried blood. For boxed trim, one MT-Mitt was used to sample the surface of the product in five boxes, while for trim combo bins, one MT-Mitt was used per combo. A total of 166 and 288 trim samples were taken from a mixture of boxes and combos in rounds 1 and 2, respectively. Boxes and combos

were utilized based on the processor's requirements. The trim in boxes or combo bins was each swabbed for a total of 90 seconds, with the MT-Mitt flipped over after 45 seconds. After sample collection, MT-Mitts were placed in sterile bags separated by replicate number and transported under refrigeration overnight to the Eurofins Microbiology Laboratory in Omaha, NE (round 1), or processed in the Eurofins plant laboratory (round 2) for microbiological testing.

3.2.2 Sample Processing

All samples were analyzed for pathogen index targets using a real-time polymerase chain reaction (PCR) detection system. Upon arrival at the laboratory, approximately 200 mL of Modified Tryptic Soy Broth (Gold Standard Diagnostics, Horsham, PA) was added to each MT-Mitt sample, followed by mechanical pummeling (Interscience BagMixer 400, Paris, France) for 1 minute. Samples were then incubated at 41.5°C for a minimum of 10 hours. Following the incubation, sample enrichments were shipped to the Eurofins Microbiological Laboratories in Louisville, KY, where they were held at -20°C until they were processed for PCR analysis. For round 2, PCR analysis was performed in-house at the Eurofins plant laboratory.

3.2.3 PCR Analysis

All enriched samples were analyzed for pathogen index targets using PCR. An aliquot of the enrichments from the MT-Mitt swabs of the upper and lower sampling areas for each lot was combined into a 5mL tube for PCR analysis. From the tube, a 30 µL aliquot of the combined enrichment was added to 70 µL of pre-weighed lysis buffer, consisting of Lysis Buffer S (a proprietary buffer) and proteinase K, to generate the lysate. The lysate was heated to 37°C on heating blocks (Thermo Fisher Scientific, Waltham, MA) for 20 minutes, followed by 95°C for

10 minutes, and then allowed to cool for 5 minutes at 4°C. The lysate was then kept refrigerated (4°C) until the PCR assay.

The following commercially available BAC*Gene* real-time PCR kits were used: BAC*Gene* Mplex STEC Screen, BAC*Gene E. coli* O157:H7, BAC*Gene* Mplex STEC SEROtype 1, and BAC*Gene* Mplex STEC SEROtype 2 (Gold Standard Diagnostics, Horsham, PA). These kits were used to screen samples for the index targets shown in Table 3.1. The previously prepared lysate and the Master mix from the kit were centrifuged for 30 seconds before 5 µL of lysate was added to 20 µL of Master Mix (Fisher Scientific, Waltham, MA). Negative and positive controls, consisting of ultra-pure distilled water and plasmid from the respective PCR kit, were utilized for every run. After the Master Mix and lysate were mixed, tubes were vortexed for 15 seconds at 1150 rpm (Fisherbrand™ Scientific Analog vortex) and then centrifuged for 30 seconds using a Fisherbrand™ Microplate Centrifuge (Fisher Scientific, Waltham, MA). Samples were then run in a Bio-Rad CFX96 Deep Well™ Real-Time PCR machine (Bio-Rad Laboratories, Inc., Hercules, CA) or an AriaMX Real-Time PCR machine (Agilent Technologies, Inc., Santa Clara, CA). Data were analyzed using PURE software version 1.03 for the STEC and O157 panels, version 1.01 for STEC serotype one, and version 1.02 for STEC serotype two (Gold Standard Diagnostics, Horsham, PA). Samples that had a value were presumed positive for the respective pathogen indicator.

3.3 Results

Prevalence data for the pathogen index targets evaluated in round 1 of Phase 2, for both carcasses and trim, are shown in Table 3.2. A total of 1,040 carcasses were sampled, comprising 260 MT-Mitt samples (130 mitt samples each for the upper and lower carcass sampling area) with eight carcasses per lot, over 13 consecutive production days. An aliquot of the sample

enrichments from the upper and lower MT-Mitt swabs for each lot were taken and evaluated together, which resulted in 130 MT-Mitt samples being evaluated for pathogen index targets. A total of 166 trim samples were collected, which corresponded to the carcasses that were sampled 48 hours previously. A lot consisted of either five boxes OR one trim combo bin (~approximately 2000 lb). The number of samples collected from beef trimmings each day ranged from 9-22 samples. This wide range occurred as the amount of final product produced varied each day, and a mixture of boxes and combos was utilized based on the processor's requirements.

All samples were evaluated through PCR for eight pathogen indicator targets (STEC, O157, O111, O103, O26, O145, O121, and O45; Table 3.1). For a sample to be identified as a presumptive positive for STEC, the *stx*$_1$ or *stx*$_2$ gene needed to be present with the *eae* gene. A presumptive positive for O157 means that O157 was detected along with H7, and for the rest of the indicators, any value above zero indicated a presumptive positive. For this phase, a presumptive positive was treated as positive and will be referred to as such going forward.

Out of the eight indicators evaluated, only two out of the 130 carcass samples tested positive (Table 3.1). These carcass samples, collected on days 4 and 8, tested positive for the O45 and STEC indicator, respectively, while all other samples tested for pathogen indicators over the 13 days were negative. On the other hand, there were 11 out of 16 trim samples that tested positive on day 4 for the O45 indicator, and none of the trim samples from day 8 tested positive for STEC. Out of the 166 beef trim samples evaluated, 48 individual samples were identified as positive for at least one of the eight indicators across the 13 days. There were 76 total identified positive cases, as some samples tested positive for multiple indicators. For STEC and O157, there were 37 positive cases coming from 28 samples, with 11 positives for STEC and 26 positives for O157. Out of the non-O157 indicators, O45 had the highest positive rate

(20.5%), with 34 out of the 166 trim samples being positive. For O26 and O121, four out of 166 (2.4%) and one out of 166 (0.6%) samples were positive, respectively. There were no positives identified for any of the trim samples for the O103, O145, and O111 indicators.

When prevalence data were broken down by day, no positives were identified on days 2, 11, or 12 for either carcasses or trim. The highest number of positives occurred on days 3 (18 positives) and 4 (33 positives), and all positives (except for one carcass sample on day 4) were from beef trim samples. There were 22 trim samples collected on day 3, and out of those, 9.1%, 40.9%, 13.6%, and 18.2% were positive for STEC, O157, O26, and O45, respectively. On day 4, 16 trim samples were collected, and 50%, 75%, 6.3%, and 68.8% were positive for STEC, O157, O121, and O45, respectively.

During round 2 of data collection, a total of 2,048 carcasses were sampled, with eight carcasses per lot, resulting in 512 MT-Mitt samples (256 mitts each for the upper and lower carcass sampling area). Similar to round 1, the sample enrichments of the upper and lower carcass sampling area swabs were combined, resulting in 256 carcass MT-Mitt samples being evaluated. A total of 288 trim samples were collected, corresponding to the carcasses that were sampled 48 hours previously. A lot of trim samples consisted of one combo bin. The number of samples collected from beef trimmings each day ranged from 76 to 114, and the number of samples collected from beef carcasses ranged from 80 to 90. The range of samples for both carcasses and trim was dependent upon customer and processor needs. For round 2 of sampling, no carcass or trim samples tested positive for STEC (*stx*$_1$ or *stx*$_2$ indicator with the *eae* indicator). These indicators can be present in non-pathogenic organisms; therefore, a lack of both the *stx* and *eae* indicators results in a negative test result. Due to this, the prevalence of positives was not reported; instead, the detection of the individual *stx*$_1$, *stx*$_2$, and *eae* targets was utilized as a

comparison of pathogen index target pickup between the MT-Mitt and trim samples (Table 3.3). A detectable target was represented by any sample that had a numerical value for a specific indicator.

Over the three collection days in round 2, a total of 21 targets were identified across all carcass samples, with seven targets detected on each day (Table 3.3). For the trim samples, 13 targets were identified on days 1 and 2, and five targets were identified on day 3 (Table 3.3). There were no carcasses or trim samples that had more than one indicator present (e.g., stx_1 and stx_2). When target prevalence is compared between beef carcasses and trim, there was a higher prevalence on days 1 and 2 for trim, but on day 3, carcasses had a higher target detection prevalence. The lack of consistency in results in this round also indicates the need for additional testing to verify the MT-Mitt on carcasses.

3.4 Discussion

In the current study, there was a higher positive rate for trim compared to carcasses for all eight pathogen index targets evaluated in round 1. Previous research has validated the use of the MT-Mitt on beef trim using pathogen index targets. The MT-Mitt was compared with the CSD, MSD, and MT-Swab on beef trim, and there were no significant differences between the MT-Mitt and any other sampling method when evaluated for the intimin, hemolysin, O group, and H7 genes (Arthur and Wheeler, 2021; Arthur et al., 2024). Although no previous studies have investigated the use of the MT-Mitt on carcasses, other cloth sampling methods using different materials on carcasses have been investigated. A cheesecloth, sponge, and swab were evaluated against excision on carcasses, and all of them were found to be less effective in recovering bacteria than the excision method (Dorsa et al., 1996). These authors used uninoculated and inoculated carcasses and found the same result for both categories; however, they did not use pathogen index targets. A separate study investigated the prevalence of *E. coli* O157:H7 and

Salmonella on cattle hides and carcasses using pathogen index targets analyzed by PCR, and identified pathogen targets on carcasses (Brichta-Harhay et al., 2008). This study used a similar swabbing method as the current phase and sampled the same general areas of carcasses as the current study; however, the swabs were pre-wetted with sterile buffered peptone water before use (Arthur et al., 2004; Brichta-Harhay et al., 2008). Neither of the studies compared results to trim; however, they had positive samples while swabbing carcasses, unlike in the current study, which had very limited positive results from the carcass swabs. As reported in Chapter 2, we were able to recover aerobic bacteria from the carcasses while using the MT-Mitts, indicating that the mitts can effectively pick up bacteria from the carcass surface.

The results from this phase, specifically round 1, differed from expectations based on prior research and general understanding of initial pathogen contamination. The MT-Mitt sampled a much larger surface area on the carcasses when compared to sampling the top layer of a 2000 lb. trim combo. Therefore, there is a greater chance of recovering pathogen index targets if they are present on the carcass. This data exhibited the opposite trend, with MT-Mitt trim samples detecting more pathogen index targets than MT-Mitt carcass samples. Although the results differed from the anticipated results, several factors may explain these differences. In round 1, the data were collected in a commercial beef processing plant, harvesting only 80 animals a day over approximately seven hours. The plant also utilized hot water and lactic acid as final interventions. However, at a slow line speed, carcasses were exposed to these interventions for a much longer time than they would be at a faster line speed. This meant it took upwards of 30 minutes to collect a single sample lot (eight carcasses), during this time the lactic acid could have continuously been active on bacteria already picked up on the mitt surface. To mitigate the potential effects of the interventions on the sampling mitt, we tested the addition of a

neutralizing buffer on days 8 and 9 and turning the lactic acid cabinet off on days 11-13; however, this resulted in minimal changes in the outcomes. Another possibility is that the pathogen detection might have been limited by the sampled area, though this is unlikely. The MT-Mitt provides broad carcass coverage, potentially leaving only a small central section unsampled. Previous research has shown that the highest probability of contamination corresponds to initial skinning cuts and hide removal pattern lines, particularly around the brisket, shank, and neck near the stick wound (Reid et al., 2002; Antic et al., 2010). Conscious efforts were made to ensure the sampling of these areas in the current study, suggesting that the sampling location was not a contributing factor to the limited pathogen target detection on carcass swabs. In round 2, data were closer to what was anticipated based on prior research, which could be attributed to a faster line speed and less time spent in the lactic acid and hot water cabinets.

3.5 Conclusions

The results from the current phase showed a lack of agreement between the results for the carcasses and trim. Various factors could have contributed to the results in this phase, such as a slow line speed and excessive time carcasses spent in the lactic acid cabinet. Data collected at the second visit showed more similar target prevalence rates. Due to inconsistencies between both rounds of data collection and a lack of similarity between carcass and trim results, more research may be needed to validate the MT-Mitt for carcass sampling.

Table 3.1. Indicators evaluated in each of the four BAC*Gene* kits utilized.

PCR Kit	**Indicators Identified**
BAC*Gene* Mplex STEC Screen	*stx*$_1$, *stx*$_2$, and *eae*
BAC*Gene E. coli* O157:H7	O157 (excluding H7) or O157:H7 combined
BAC*Gene* Mplex STEC SERO*type1*	O111, O103, and O26
BAC*Gene* Mplex STEC SERO*type2*	O145, O121, O45

Table 3.2. Pathogen indicator target prevalence (%) for carcass and trim samples (round 1) using the MT-Mitt across 13 consecutive production days in a commercial beef processing plant.

Indicator	Sample Type	Day													
		1	2	3	4	5	6	7	8	9	10	11[1]	12[1]	13[1]	Total positive samples
STEC[2] (*stx*$_1$, *stx*$_2$, and *eae*)	**Carcass**	**0%**	**0%**	**0%**	**0%**	**0%**	**0%**	**0%**	**10% (1/10)[3]**	**0%**	**0%**	**0%**	**0%**	**0%**	**1**
	Trim	0%	0%	9.1% (2/22)	50% (8/16)	0%	0%	0%	0%	11.1% (1/9)	0%	0%	0%	0%	11
O157	**Carcass**	**0%**	**0%**	**0%**	**0%**	**0%**	**0%**	**0%**	**0%**	**0%**	**0%**	**0%**	**0%**	**0%**	**0**
	Trim	0%	0%	40.9% (9/22)	75% (12/16)	8.3% (1/12)	0%	0%	0%	22.2% (2/9)	0%	0%	0%	22.2% (2/9)	26
O111	**Carcass**	**0%**	**0%**	**0%**	**0%**	**0%**	**0%**	**0%**	**0%**	**0%**	**0%**	**0%**	**0%**	**0%**	**0**
	Trim	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0
O103	**Carcass**	**0%**	**0%**	**0%**	**0%**	**0%**	**0%**	**0%**	**0%**	**0%**	**0%**	**0%**	**0%**	**0%**	**0**
	Trim	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0
O26	**Carcass**	**0%**	**0%**	**0%**	**0%**	**0%**	**0%**	**0%**	**0%**	**0%**	**0%**	**0%**	**0%**	**0%**	**0**
	Trim	0%	0%	13.6% (3/22)	0%	0%	0%	12.5% (1/8)	0%	0%	0%	0%	0%	0%	4
O145	**Carcass**	**0%**	**0%**	**0%**	**0%**	**0%**	**0%**	**0%**	**0%**	**0%**	**0%**	**0%**	**0%**	**0%**	**0**
	Trim	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0
O121	**Carcass**	**0%**	**0%**	**0%**	**0%**	**0%**	**0%**	**0%**	**0%**	**0%**	**0%**	**0%**	**0%**	**0%**	**0**
	Trim	0%	0%	0%	6.3% (1/16)	0%	0%	0%	0%	0%	0%	0%	0%	0%	1
O45	**Carcass**	**0%**	**0%**	**0%**	**10% (1/10)**	**0%**	**0%**	**0%**	**0%**	**0%**	**0%**	**0%**	**0%**	**0%**	**1**
	Trim	18.8% (3/16)	0%	18.2% (4/22)	68.8% (11/16)	25% (3/12)	33.3% (4/12)	0%	18.2% (2/11)	44.4% (4/9)	10% (1/10)	0%	0%	22.2% (2/9)	33

[1] Lactic acid cabinet was turned off on days 11-13 of sampling.
[2] The STEC target evaluated samples for three genes (*stx*$_1$, *stx*$_2$, and *eae),* and a positive result was achieved by the presence of the *stx*$_1$ or *stx*$_2$ gene AND the *eae* gene.
[3] Number of presumptive positive samples/total number of samples for that day.

Table 3.3. Shiga toxin-producing *E. coli* (STEC) pathogen indicator target prevalence (%) for carcass and trim samples (round 2) using the MT-Mitt across 3 consecutive production days in a commercial beef processing plant.

Indicator	**Sample Type**	**Day**		
		1	2	3
STEC[1] (*stx*$_1$, *stx*$_2$, and *eae*)	**Carcass**	**8.8% (7/80)[2]**	**8.1% (7/86)**	**7.8% (7/90)**
	Trim	13.3% (13/98)	11.4% (13/114)	6.6% (5/76)

[1] The STEC indicator evaluated samples for three genes (*stx*$_1$, *stx*$_2$, and *eae*)
[2] Number of targets identified/total number of samples for that day

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