INCIDENCE OF LISTERIA SPECIES IN CATTLE HOLDING FACILITES, FECES, HIDES AND FINISHED BEEF CARCASSES

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CHAPTER 1

Literature Review

Introduction

During the past twenty years, *Listeria monocytogenes* has emerged as significant food borne pathogens. *Listeria monocytogenes* has been identified as a human pathogen since the 1920's (Schlech, 2000). However, food borne transmission was not implicated until 1981, when Schlech et al. (1983) reported the isolation of *Listeria monocytogenes*, serotype 4b from coleslaw that had been consumed by a patient that had an infection with serotype 4b.

General Characteristics of *Listeria monocytogenes*

The genus *Listeria* is a Gram-positive bacteria consisting of 6 genera: *L. monocytogenes*, *L. ivanovii*, *L. innocua*, *L. welshimeri*, *L. grayi* and *L. seeligeri*. While *L. monocytogenes* is not the only *Listeria* spp. capable of causing infection, *L. monocytogenes* is responsible for virtually all cases of foodborne human listeriosis (Ryser and Marth, 1991). *L. monocytogenes* can grow at temperatures between 1 and 45°C with an optimum growth temperature between 30 and 37°C. It is capable of growth between pH 6 and 9, and can grow in nutrient broth supplemented with up to 10% (w/v) NaCl. Although *L. monocytogenes* is an aerobic, mesophilic organism, it is capable of growing well when some oxygen is replaced with carbon dioxide, but it will not grow under strict anaerobic conditions.

All *Listeria* spp. possess the following biochemical traits: catalase (+), oxidase (-), urease (-), and esculin and sodium hippurate are hydrolyzed. However, species differentiation can be made, in part, based upon acid production from mannitol, rhamnose, and xylose, as well as the CAMP test using *Staphylococcus aureus* and *Rhodococcus equi*. *L. monocytogenes* is capable of fermenting rhamnose but cannot ferment xylose or mannitol. It is β-hemolytic on sheep blood agar, and hemolysis is enhanced by the *S. aureus* CAMP factor.

Further identification of *L. monocytogenes* can be performed using serotyping. There are currently 13 serotypes of *L. monocytogenes*. Approximately 90% of the clinical isolates belonging to serotypes 1/2a, 1/2b, and 4b. The remaining serotypes are 1/2c, 3a, 3b, 3c, 4a, 4ab, 4c, 4d, 4e, and 7. There are also various somatic (O) and flagellar (H) structures that can be used in serotyping (Ryser and Marth, 1991). Other identification and characterization procedures may include use of the Polymerase Chain Reaction, DNA fingerprinting, phage-typing, and ribo-typing.

Persistence of *L. monocytogenes* within the Animal Production Environment

Listeria monocytogenes presents a unique concern for the processor of ready-to-eat (RTE) meats, because it is not considered an adulterant in raw meat products but is considered an adulterant of finished RTE products. However, this organism can present a concern from the standpoint of it entering the processing environment at the time of slaughter (Fenlon et al., 1996). Another concern is

dissemination through the agriculture production environment. An example of this dissemination would be if this organism was being shed in animal feces and the manure was then used as fertilizer. Vegetables could become contaminated with *Listeria monocytogenes* (Van Renterghen et al., 1991) and cause illness that could potentially be traced back to the production facility (Schlech et al., 1983).

Van Renterghen et al. (1991) studied the prevalence of *L. monocytogenes* within the agriculture ecosystem. They examined the prevalence of *Listeria* spp. in environmental samples from pig and cattle feces as well as manure piles, soil and ground water. *Listeria* spp. was found in 16% (4 of 25) and 20% (5 of 25) of pig and cattle feces samples, respectively, while no *Listeria* spp. were isolated from 10 manure or 17 soil samples. However, one of five ground water samples was positive for *Listeria* spp. The author also examined the survivability of *L. monocytogenes* when inoculated into cattle and pig feces, as well as soil. In experimentally inoculated pig feces, *L. monocytogenes* was able to survive between 3 and 4 weeks, while in cattle feces, *L. monocytogenes* was not recovered after 8 weeks.

Husu (1990b) investigated 249 dairy herds for shedding of *Listeria* spp. A total of 3,878 fecal samples were collected, of which 373 (9.6%) were positive for one or more *Listeria* spp. On a herd basis, at least one animal was shedding *Listeria* spp. in 114 of the 249 (45.8%) herds sampled. On average, 2.12% of the animals in any given herd were shedding *Listeria* spp. In a second phase of the study, fecal, milk and feed samples were collected from 80 dairy farms and examined for *Listeria*. A total of 314 fecal samples were collected, with *L.*

monocytogenes and *L. innocua* isolated from 68 (21.7%) and 23 (7.3%) samples, respectively. A total of 314 tank milk samples were collected, of which 7 (2.2%) and 6 (1.9%) were positive for *L. monocytogenes* and *L. innocua*, respectively. When 295 feed samples (grass silage or pasture grass) were tested, *Listeria monocytogenes* was recovered from 62 (21.2%) and *L. innocua* was recovered from 33 (11.2%) samples. Burrow et al. (1996) found higher numbers of *L. monocytogenes* present in animal feces, as 33% of the cattle, 8% of the sheep, 5.9% of the swine and 8% of the hens they sampled were found to be positive.

In a second study, Husu et al. (1990a) investigated 4 farms for the presence of *Listeria* spp. The study revealed that 4 of 59 fecal samples (6.8%) were positive for *Listeria monocytogenes* while 3 of 59 (5.1%) were positive for *L. innocua*. Feed samples were collected, including silage, hay concentrates and other feed ingredients. *Listeria* spp., either *L. innocua* or *L. monocytogenes* was isolated from 1 of 8 feed samples, 5 of 8 hay samples, 8 of 8 oat meal or crossed oats samples, 2 of 2 oat mash and 2 of 2 straw samples. Environmental samples that were *Listeria* spp. positive (*L. innocua* or *L. monocytogenes*) included the feed passage (14 of 16), water cups (24 of 46), walls (4 of 16), floors (12 of 16) and windows (1 of 8).

While dairy farms have been a major focus of the *Listeria* research conducted in the agricultural ecosystem primarily due to the risk of milk being contaminated by *Listeria monocytogenes*, the feedlot environment has also been investigated to better understand the survival of this organism within the animal production environment. Siragusa et al. (1993) collected both composite and

individual fecal grab samples over a 3-month period. A total of 69 composite fecal samples were collected with 14 (20%) positive for either *L. innocua* or *L. welshimeri* and one sample (1.4%) was positive for *Listeria monocytogenes*. In addition to the composite samples, a total of 138 individual samples were collected, with 13 (9.4%) cattle positive for *L. innocua* or *L. welshimeri* and again, 1 sample positive for *L. monocytogenes*.

In this study, Siragusa et al. (1993) also investigated the impact of a silage diet on the shedding of *Listeria.* Initially, 23 cattle were being fed a grass diet, and no *Listeria* were isolated. However, when these cattle were switched to a silage diet, *Listeria monocytogenes* was isolated from 8 of 27 cattle and additional *Listeria* species were isolated from another 8 animals. Electrophoretic typing (ET) revealed the same *L. monocytogenes* isolate was recovered from both silage and cattle. These results are consistent with other research that has linked a silage diet to the shedding of *Listeria* spp. (Siragusa et al., 1993).

Fenlon et al., (1996) performed an extensive investigation into *Listeria* and the livestock production environment that included avian fauna, farm ducks and domestic farm animals. Of the 23 samples collected, 3 (13%) were found to be shedding *Listeria monocytogenes* and 5 (21.7%) were shedding *Listeria innocua*. Grass and silage were also sampled, and while no *L. monocytogenes* was isolated from the grass pre-harvest, 3 of 10 samples were positive for *L. innocua and L. seeligeri*. However, within 24 hours of harvesting the grass (to make silage) 9 of the 10 samples collected were positive for *L. monocytogenes*.

Listeria spp. has been isolated from animals in the production environment in a range of 9.6% to 33% incidence. However, little research has focused upon factors that may be associated with the shedding and dissemination of this organism. Silage, when not fermented properly, appears to be a source of *Listeria monocytogenes* (Husu et al., 1990a). However, other sources may need to be considered, such as other feedstuffs, or equipment that is used within the production facility. Another phenomenon observed in research is that *Listeria innocua* is found as often, or more than, any other *Listeria* spp. in the production environment (Siragusa et al., 1993).

Factors Associated with Preharvest and Postharvest Carcass Contamination

At the time of slaughter, a processor is faced with multiple carcass contamination concerns. Research has demonstrated that the stress associated with shipping livestock from the production facility to the slaughter facility can increase the shedding of pathogens in the feces (Fenlon et al., 1996). Other sources of contamination that must be considered include hide and hair (Korsak et al., 1998) and equipment used by employees such as knives, gloves and aprons (Reagan et al., 1996). Larger pieces of equipment such as those used for hide removal and carcass breaking can also present contamination concerns (Gill and Jones, 1999b).

When cattle are shipped to slaughter, they undergo a great deal of stress. If *L. monocytogenes* have already colonized the intestinal tract of these animals,

they may begin shedding these pathogens in their feces. Fenlon et al. (1996) investigated the shedding of *L. monocytogenes* before and after transport to slaughter, transportation as well as the effect that distance may have on the shedding of this organism. The slaughter facilities that were used for the study were either less than 25 km or greater than 125 km from the production facility. The author found that, in cattle traveling less than 25 km, the incidence of shedding was not significantly increased. However, when transported greater than 125 km, shedding increased significantly. In research with *Salmonella typhimurium*, Puyalto et al., (1997) were able to demonstrate an increased shedding of this organism during shipping. Prior to shipping, 8% of the cattle being transported to slaughter had *S. typhimurium* in their feces. However, after arrival at the slaughter facility, 25% of the animals were shedding this organism.

Increased shedding of pathogens in the feces as well as mud and manure (commonly referred to as 'tag') that may be attached to the hide can present contamination risks. Donkersgoed et al. (1998) investigated the association of tag with carcass contamination by enumerating total bacteria by aerobic plate count (APC), total coliforms, and *E. coli*. The authors found no consistent association of tag with bacterial contamination of carcasses, as removing tag or slowing the line speed resulted in minimal reductions of <0.5 $\log_{10}$ CFU/cm^2 for APC, coliforms and *E. coli*. Aerobic plate count results by McEvoy et al., (2000) comparing 'clean' cattle to 'dirty' cattle showed carcasses from animals with little to no tag had between 0.53 and 0.62 fewer $\log_{10}$ CFU/cm^2 than carcasses from animals with excessive tag. This was also similar to the results found by Ridell

and Korkeala (1993), in which cattle with excessive tag had an average 0.68 $\log_{10}$ CFU/cm^2 higher bacterial counts.

Elder et al. (2000) investigated the incidence of *E. coli* O157:H7 on the hide and in the feces of cattle at the time of slaughter, as well as sampling of carcasses pre- and post-evisceration and post-processing. A total of 327 fecal samples and 355 hide samples were collected with 91 (27.8%) and 38 (10.7%) positive isolates, respectively. Pre-evisceration carcass swabs identified 148 of 341 (43.4%) positive carcasses. Only 59 of 332 (17.8%) carcasses were positive post-evisceration and 6 of 330 (1.8%) were positive post-processing. Of the carcasses that were positive, 95 were positive only pre-evisceration while 47 were positive both pre-evisceration and post-evisceration, and 10 carcasses were only positive post-evisceration. All 6 of the post-processing positives were positive post-evisceration and three were positive pre-evisceration.

The potential for carcass contamination from the hide and feces presents a problem that several approaches tried to address. Several different procedures have been utilized in an attempt to remove tag from the hide of the animal. A simple approach has been a pre-slaughter washing of the animal prior to stunning. Another, more complex system, has consisted of chemically dehairing the animal after stunning, prior to bleeding. Byrne et al. (2000) inoculated 30 heifers with 200 ml of manure inoculated with *E. coli* NCTC 12900 that was streptomycin sulphate resistant. The fecal inoculum was allowed to dry for 24 hours on the cattle prior to slaughter. The cattle were then slaughtered, with 10 head unwashed, 10 head washed for 1 minute and 10 head washed for 3

minutes utilizing a power hose at a pressure of 150 psi. The inoculum level dropped significantly after 24 hours from an initial $6.6x10^3$ CFU/cm^2 to $1.9x10^2$ CFU/cm^2. After the 1 minute wash there was no significant change in the level of *E. coli* present on the hide. However, after the 3 minute wash the level of *E. coli* had been significantly reduced to 2.3 CFU/cm^2. When finished carcasses were sampled, the control averaged 8.13 CFU/cm^2 while the 1 and 3 minute washes averaged 2.07 and 0.29 CFU/cm^2, respectively.

Chemically removing the hair from cattle prior to initiating hide removal has also been investigated as a method to reduce carcass contamination. Schnell, et al. (1995) examined the effects of dehairing on the bacterial counts of the carcass, overall carcass cleanliness and trimming required to meet zero tolerance requirements. Carcasses were dehaired using a method patented by Bowling and Clayton (1992). The dehairing solution was a 10% sodium sulfide solution while 3% hydrogen peroxide was used as a neutralizer. No significant difference was found between conventional slaughtered and de-haired cattle for aerobic plate count and *E. coli*. The dehaired carcass had lower APC (4.00 log CFU/cm^2 versus 4.14 log CFU/cm^2) and *E. coli* counts (1.21 log CFU/cm^2 versus 1.14 log CFU/cm^2) but had higher total coliform counts (1.96 log CFU/cm^2 versus 1.64 log CFU/cm^2) than the conventional slaughtered carcasses. Also, *Listeria monocytogenes* was isolated from both a conventionally slaughtered animal and a dehaired animal, but no *Salmonella* were recovered from either. Overall, dehairing did increase the visual cleanliness of the carcass, but it did not reduce the microbial contamination of the finished carcasses.

Results by Castillo et al., (1998a), differ considerably from those of Schnell et al. (1995). In this study, pieces of hide were collected from the abattoir and coated with feces inoculated with rifampicin resistant *S. typhimurium, E. coli* O157:H7, or uninoculated feces. Hide samples were treated with the same process used by Schnell et al. (1995). Aerobic plate counts, coliforms and *E. coli* were enumerated, as well as *S. typhimurium* and *E. coli* O157:H7. Dehairing resulted in a 3.4 $\log_{10}$ CFU/cm^2 reduction of APC, a 3.9 $\log_{10}$ CFU/cm^2 reduction of coliforms and a >4.3 $\log_{10}$ CFU/cm^2 reduction of *E. coli*. *S. typhimurium* was reduced by >4.6 $\log_{10}$ CFU/cm^2 and *E. coli* O157:H7 was reduced by >4.8 $\log_{10}$ CFU/cm^2. It should be noted however, that artificial fecally contaminated carcasses may not have the tenacity as natural tag which could indicate better resultes than could actually be achieved in the processing facility.

These studies demonstrate that either washing or dehairing of hides could be useful in reducing the microbial load borne by the animal at the time of slaughter and hide removal. The dramatic differences between Castillo et al. (1998a), and Schnell et al. (1995) are not expounded upon in the literature. However, Schnell et al. (1995) did state several possibilities as to why there were no significant differences between the conventional and dehaired carcasses. The authors stated that the facility was not designed to process dehaired cattle, therefore aerosol, human and equipment contamination could have contributed to contaminating the carcasses. It was also observed that de-haired cattle required more handling and hide removal was complicated because the hide was ‘slippery and soapy’ from the dehairing process. Even though the authors’

research did not demonstrate a significant reduction in the bacterial load of the carcasses, the authors' did theorize that, over a period of time, in a properly designed facility, the removal of dirt and fecal matter from the hide should be useful in reducing carcass contamination with pathogens.

While the hide of the animal can be a source of carcass contamination, it is also possible for carcasses to become contaminated during subsequent breaking procedures. The carcass can become contaminated from equipment such as saws and knives or through contact with hands or protective equipment worn by the plant employee (Gill and Jones, 1999b). Gill and Jones investigated the carcass breaking process, and randomly selected and swabbed 25 carcasses and enumerated aerobic bacteria, coliforms and *E. coli*, then re-swabbed the loins after the carcass breaking process had been completed. The average aerobic count on the carcasses was 2.01 $\log_{10}$ CFU/cm^2, while coliforms and *E. coli* were not detectable from 24 and 25 of the samples, respectively. However, after breaking, the loins had mean aerobic counts of 2.15 $\log_{10}$ CFU/cm^2, coliforms averaged 1.92 $\log_{10}$ CFU/cm^2 and *E. coli* counts averaged 1.70 $\log_{10}$ CFU/cm^2.

Further investigation by Gill and Jones (1999b) found that approximately 5% of the carcasses entering the breaking facility had *E. coli* counts >2 $\log_{10}$ CFU/cm^2 on the anal region. They also found that procedures such as sawing through the backbone, removing the ribs and sternum and injecting nitrogen between the shoulder muscles could increase aerobic counts, but did not significantly influence coliform counts. Other trimming procedures were not

found to significantly impact the microbial load of the carcass, with the exception of the anus region, in which they found that trimming could reduce coliforms and *E. coli* by an average of approximately 1 log.

Gill et al., (1999a) investigated inadequately cleaned equipment used in the breaking of sheep carcasses. The equipment sampled included the blade and guard of the carcass saw, the forequarter table surface, the band saw table, guard and drive wheels, the meat contacting surface of conveyor belts, conveyor belt support bars, cutting boards and mesh gloves. The equipment was sampled prior to work commencing for the day. Total aerobes, coliforms and *E. coli* were enumerated from the shoulder, loin and leg, and swabs were collected before breaking, after sawing or after trimming. No significant difference in aerobic counts was observed during the breaking process, as the shoulder, loin and leg had initial log counts of 2.81, 2.80 and 2.56 CFU/cm^2, respectively, prior to breaking and 2.79, 2.57 and 3.32 CFU/cm^2, respectively, after sawing and trimming. Coliforms did not increase significantly on the shoulder during processing but both the loin and leg saw significant increases in coliforms and *E. coli* after trimming. No coliforms were recovered from the sampled equipment. However, mesh gloves, which were the responsibility of individual plant personnel to clean, had coliform and *E. coli* counts of 5.54 and 4.73 $\log_{10}$ CFU per glove, respectively. Therefore, the authors observed inadequately cleaned mesh gloves worn by plant employees can present a significant contamination source.

In another study published by Gill et al., (1999c) inadequately cleaned equipment was identified and *E. coli* was found to persist and proliferate in equipment that was considered to be well-cleaned. The authors observed that this persistence could lead to contamination of product by *E. coli* O157:H7 or other pathogenic strains of *E. coli*, if present and was supported by the increase of coliforms and *E. coli* on loins and chucks that passed through the contaminated equipment. Initial carcass swabs for coliforms and *E. coli* were below detectable levels. However, subsequent sampling of loins had an increase of coliforms and *E. coli* to 2.39 and 2.33 $\log_{10}$ CFU/cm^2 while chucks increased to 1.16 and 0.58 $\log_{10}$ CFU/cm^2, respectively.

These results led Gill and McGinnis (2000) to investigate the contamination of beef trimmings with *E. coli* from equipment and gloves. Aerobes were recovered at levels as high as 10^5 CFU/cm^2 per sample from swabs of pooled water on cleaned equipment. Steel mesh gloves and inadequately cleaned equipment had counts as high as 10^8 CFU/cm^2 per sample. Coliforms were not recovered from the water samples, and while coliforms were recovered from gloves and equipment, less than 10% of the coliforms recovered were *E. coli*. However, in meat trimmings, *E. coli* was the predominant coliform. Thus, the authors concluded that carcass contamination may occur when a localized contamination site exists, and the carcass breaking process then distributes the contamination throughout the product.

Mesh gloves can present a contamination hazard, as has already been demonstrated. Legg et al. (1999) wanted to determine if wearing neoprene

gloves would provide an advantage at reducing cross contamination when compared to using bare hands. They compared bare hands to gloved hands rinsed at 40°C and gloved hands rinsed at 60°C. The bacterial culture was obtained by swabbing hides of cattle and using that to inoculate the growth medium. A bare or gloved hand was dipped in the culture for 30 seconds, then allowed to 'drip' for 10 seconds. A 5-cm^2 area of the palm was swabbed and plated on nonselective media. The hand was then rinsed with 40° or 60°C water for 5 seconds and allowed to drip for another 10 seconds prior to another swab adjacent to the previous swab area.

The bare hand had a mean pre-rinse of 12.8 CFU/cm^2. After rinsing with 40°C water, the mean was 1.0 CFU/cm^2. The gloved hand had an initial inoculum mean of 21.3 CFU/cm^2 prior to the 40°C rinse and 23.6 CFU/cm^2 prior to the 60°C rinse. Post-rinse counts were 0.5 and 0.1 CFU/cm^2, respectively. There was no statistical difference between the three methods after rinsing, even though the inoculum level was significantly higher for the gloved hands. The author observed that this study did not account for build up of residues on the glove or bare hand or the possibility of increasing the bacterial load of the gloves throughout the day. It was also observed that the rinse water flow rate would probably have an impact upon removal of the build up.

Research has shown that carcass contamination occurs in many forms, and there is no single step that can be taken to eliminate contamination. However, it has been demonstrated that, if various steps such as removing dirt from cattle prior to slaughter, proper cleaning of equipment, and proper care of

gloves are taken throughout the slaughter process, then microbial load born by the carcass may be reduced.

Carcass Decontamination

Despite processors making various attempts to prevent carcass contamination, there is no single 'magic bullet' solution. Therefore, further steps are needed to reduce or eliminate any carcass contamination that may occur during the slaughtering process. Knife trimming has typically been the first line of defense. Also, cold and hot water rinses have been shown to reduce contamination. Other pasteurization or decontamination methods have also been investigated and include the use of very hot water (>82°C), steam vacuuming, steam cabinets, and acid washes (Phebus et al., 1997).

Phebus et al., (1997) investigated a steam pasteurization process patented by Frigoscandia Food Process Systems and compared it to other methods. In the laboratory, freshly slaughtered beef tissue was inoculated with feces containing *E. coli* O157:H7, *S. typhimurium* and *L. monocytogenes*. Treatments included trimming, washing, vacuuming and steam. Washing gave the least reduction, with a mean reduction of 0.75, 1.23 and 1.28 $\log_{10}$ CFU/cm^2 for *E. coli* O157:H7, *S. typhimurium* and *L. monocytogenes*, respectively. Trimming had reductions of 3.10, 2.72 and 2.54 $\log_{10}$ CFU/cm^2, vacuuming resulted in reductions of 3.11, 3.37 and 3.33 $\log_{10}$ CFU/cm^2 and steam gave reductions of 3.53, 3.74 and 3.44 $\log_{10}$ CFU/cm^2 for *E. coli* O157:H7, *S. typhimurium*, and *L. monocytogenes,* respectively.

Phebus et al., (1997) also compared combined treatments of trimming-washing-2% lactic acid spray-steam pasteurization to vacuuming-washing-2% lactic acid spray-steam pasteurization and found no significant difference between the two sets of decontamination steps for all three pathogens, as reductions between 4.14 to 5.31 $\log_{10}/cm^2$ were obtained. The author concluded that, while trimming and vacuuming have the potential to reduce microbial contamination, they depend upon visible contamination to be effective. Lactic acid and steam were found to give comparable results, but it was pointed out that lactic acid may corrode equipment and could present more of a safety hazard to employees, problems that were not found with a steam cabinet.

Nutsch et al. (1998) further investigated this process by sampling 40 carcasses before treatment and 40 carcasses post treatment. Before treatment, the midline had the highest aerobic plate count (4.5 $\log_{10}/100\ cm^2$). The inside round, brisket and neck had average counts of 3.8 $\log_{10}/100\ cm^2$ and the loin averaged counts of 3.4 $\log_{10}/100\ cm^2$. After treatment, all counts were reduced significantly, as the round, loin and brisket had counts of approximately 2.6 $\log_{10}/100\ cm^2$ and the midline and neck had counts of 3.1 and 3.3 $\log_{10}/100\ cm^2$, respectively. Generic *E. coli* populations were low throughout the study. However, before treatment, 32% of the carcasses had *E. coli* counts below the detectible limit of 5.0 CFU/100 cm^2, whereas after pasteurization, 85% of the carcasses had *E. coli* populations below the detectible limit.

Cutter et al., (1997) investigated multiple aspects related to using a 2% acetic acid solution compared to water. Prerigor, postrigor, and frozen beef

carcass tissue gave similar results when inoculated with *E. coli* O157:H7 mixed into sterile feces. All three meat types had between a 1.74 and 2.23 $\log_{10}$ reduction using either water or acetic acid solution. The research also examined the difference between the attachment and survival of cells suspended in physiological saline and beef feces. While the feces gave slightly higher inoculum levels (4.87 vs. 4.53 $\log_{10}$ CFU/cm^2), than cells suspended in saline, the feces resulted in a better reduction of inoculum when washed with water, with a reduction of 2.8 $\log_{10}$ CFU/cm^2 compared to 1.7 $\log_{10}$ CFU/cm^2 reduction for the saline. Similar results were observed for the acid wash, as the saline inoculum had a 2.8 $\log_{10}$ reduction while the fecal inoculum had a 3.0 $\log_{10}$ reduction. However, no statistical differences were observed between the two methods.

Cutter and Siragusa (1994) compared various concentrations (1%, 3% and 5%) of lactic acid, acetic acid and citric acid on reducing a 3 strain mixture of *E. coli* O157:H7 attached to inoculated beef carcass tissue. No statistical difference was observed between acid types. However, concentration and tissue type were observed to be significant, as adipose tissue gave greater $\log_{10}$ reductions of *E. coli* O157:H7 than did lean tissue, and increasing the concentration of the acid also gave the greatest reductions.

Dorsa et al. (1997) evaluated water, 1.5% and 3.0% glacial acetic acid, 1.5% and 3% lactic acid and 12% trisodium phosphate (TSP) for the ability to reduce levels of *L. innocua*, and *E. coli* O157:H7 on inoculated tissue, as well as determine the effectiveness of the treatments over 21 days. Total aerobic

bacteria were initially reduced by 1.3 to 2.0 log CFU/cm^2 by all wash treatments. However, after 2 days, the water and TSP washed tissue total plate count had increased by greater than 1 log cycle and after 21 days, the population had increased to 8 log_{10} CFU/cm^2 while the control had increased to 8.5 log_{10} CFU/cm^2. All acid treatments resulted in an initial reduction of approximately 1.5 log_{10} CFU/cm^2. After 21 days, a population of 5.5 log_{10} CFU/cm^2 was observed. This was significantly less than the untreated controls, water or TSP.

The acid and TSP reduced *E. coli* O157:H7 to below detectible levels (1.3 log_{10} CFU/cm^2) from an initial level of 4.0 log_{10} CFU/cm^2. After 21 days the control had increased to 5.8 log_{10} CFU/cm^2, while all treated samples (except water) remained undetectable. The water washed sample was initially reduced by 1.8 log cycles, and after 21 days, the water washed sample was still 1.5 log lower than the control. Similar results were observed with *L. innocua* with the exception of TSP and water. Initially, *L. innocua* was reduced by >4 log cycles for all 4 acid treatments to undetectable levels. The control increased to >8 log CFU/cm^2 after 21 days while the acid treated samples remained undetectable. The population of TSP treated samples was significantly less than the population of the untreated or water washed sample after 21 days, although some growth was observed.

Dorsa et al. (1998a) observed similar results in a study using beef shortplates. Using a low inoculum (<2 log_{10}) of *L. innocua*, *E. coli* O157:H7 and *S. typhimurium*, growth was either suppressed or no organisms were recovered from lactic or acetic acid treated samples. TSP also exhibited some growth

suppression, but not to the extent of the acid treatments. Another study by Dorsa et al., (1998b) was performed on meat that was inoculated, treated and then ground and stored for 21 days. Treatments included water, 95°C hot water, 2% lactic acid, 2% acetic acid and 12% trisodium phosphate. No significant reduction was observed for either total lactic acid bacteria or aerobic mesophils at initial treatment or during the 21 day trial. However, *E. coli* O157:H7 and *L. innocua* were significantly reduced by the acid and TSP washes both initially and during storage. The hot water wash did reduce both organisms, as reductions were statistically significant, but the reduction was not as good as with the antimicrobial agents.

Water wash, trimming and combined hot water and 2% lactic acid treatments were compared by Castillo et al. (1998b) for the reduction of *E. coli* O157:H7 and *S. typhimurium*. Hot water (95°C) gave reductions from 4.0 to >4.8 $\log_{10}$ CFU/cm^2 While the 2% lactic acid spray gave reductions from 4.6 to >4.9 log CFU/cm^2. In combination, lactic acid spray followed by hot water, or hot water followed by lactic acid spray, gave reductions similar to those observed for the individual treatments.

Dickson et al. (1994) investigated the use of 8%, 10% and 12% trisodium phosphate and found no significant difference between these concentrations. Reductions of 1 to 1.5 log cycles were observed for both *E. coli* O157:H7 and *S. typhimurium* on lean tissue. Greater reductions were observed with adipose tissue, as reductions between 2 and 2.5 log cycles were obtained. Other research demonstrating significant reductions of *E. coli* O157:H7 have been

obtained with 3% hydrogen peroxide and 0.1% chlorhexadine, providing 4-$\log_{10}$ and 5-$\log_{10}$ reductions, respectively. However, 5% acetic acid was the least effective in this study, as it only gave a 1-$\log_{10}$ reduction (Delazari et al., 1998).

Research has shown that washing with water can be an effective first step in reducing carcass contamination and the beneficial effects of the initial reduction of bacteria should not be lost over time (Dorsa et al., 1998a). However, research has also demonstrated the effectiveness of steam as well as antimicrobial compounds such as lactic acid, acetic acid, citric acid and TSP in reducing food borne pathogens on inoculated meat products Tissue type has also been shown to be an important variable, as lean tissue consistently gives a lower reduction than does adipose tissue. While reductions of 1 to 2 log cycles may not be sufficient to meet zero tolerance standards, organic acid spray treatments (Cutter and Siragusa, 1994) and TSP (Dickson et al., 1994) could be beneficial as part of a hazard analysis critical control point program. Nutsch et al. (1998) concluded that the effectiveness of a carcass pasteurization process would only be effective if proper handling practices were followed, and if raw product was not contaminated during further processing. In addition, the FSIS Final Rule states that antimicrobial treatments such as acids, trisodium phosphate and chlorine, as well as spray-vacuum devices that apply pressurized steam or hot water, and then vacuum it off, are effective for reducing bacteria on carcasses (Anonymous, 1996).

Microbial Contamination of Raw Retail Meats

In recent years, there has been an increased concern regarding the safety and quality of meat products. In response to these concerns, the USDA-FSIS declared *E. coli* O157:H7 an adulterant in raw ground beef. Currently, the USDA-FSIS collects samples of finished ground beef at both inspected processing facilities and retail establishments and tests for the presence of this organism. As well, complying with Hazard Analysis-Critical Control Point (HACCP) has involved sampling raw product and enumerating aerobic mesophilic bacteria, coliforms, and *E. coli*. However, reducing the microbial load of the raw product has the added benefit of potentially increasing the shelf life of the finished product (Dixon et al., 1991, and Scanga et al., 2000).

Reducing the microbial load of the carcass at the time of slaughter and preventing or reducing further contamination during breaking has the potential to not only reduce overall microbial contamination but also reduce the risk of *E. coli* O157:H7 contamination. Dixon et al. (1991) investigated the effect of sanitation on increasing the shelf life of raw beef products. The author compared conventional slaughtering and fabrication techniques to the use of strict sanitary procedures, which included the use of clean garments, sterile disposable gloves, cleaned and sanitized work area and knives that were flamed with 95% ethanol. Also, cattle being slaughtered were thoroughly washed to remove tag and carcasses were treated with a 1% lactic acid spray both prior to evisceration and prior to entering the chill cooler. Finished carcasses from the conventional process had mean aerobic plate counts between 2.8 and 3.8 $\log_{10}$ CFU/cm^2

while the 'treated' carcasses had APCs between <0.2 to 1.2 $\log_{10}$ CFU/cm^2. The mean APCs for treated and control subprimals were 1.5 and 2.7 $\log_{10}$ CFU/cm^2, respectively. The mean APCs for steaks prepared from the subprimals were 2.8 and 5.3 $\log_{10}$ CFU/cm^2 for the treated and controls, respectively.

A study by Gill and McGinnis (1993) examined the change in microflora of beef from the processor to the retail display case. At the slaughtering plant, trimmings had a flora between 10^3 and 10^5 CFU/g, with 58% of the samples yielding approximately 4.0 $\log_{10} \pm 0.5$ CFU/g. A similar range was observed from the trimmings upon arrival at the retail outlet. However, 66% of the samples had counts of 3.5 $\log_{10} \pm 0.5$ CFU/g. After grinding, the samples yielded higher counts with a range of 10^4 to 10^7 CFU/g and slightly over half (58%) averaged approximately 5.0 $\log_{10} \pm 0.5$ CFU/g. Display product had a similar range, but 75% of the samples had counts of 6.0 $\log_{10} \pm 0.5$ CFU/g.

Another study by Gill et al. (1997) investigated the hygienic performance of hamburger patties at both production and retail. The author collected both frozen and chilled samples from three plants as well as four and three retail outlets, respectively. The counts for total aerobic plate count, coliforms and *E. coli* for chilled patties from the manufacturer ranged from 4.12 to 4.69, 0.66 to 1.06, and 0.22 to 0.92 $\log_{10}$ CFU/g, respectively. The frozen patties ranged from 3.93 to 4.45, 0.51 to 1.20, and 0.03 to 0.83 $\log_{10}$ CFU/g, respectively. The frozen patties from retail outlets ranged from 3.31 to 6.98, below detectable levels to 3.52, and below detectable levels to 1.28 $\log_{10}$ CFU/g, respectively. The chilled patties had levels from 4.39 to 7.36, 1.62 to 3.75 and 0.88 to 1.25 $\log_{10}$ CFU/g,

respectively. The authors concluded that the hygienic condition of ground beef could be improved through more hygienic procedures within the plant as well as retail outlets improving the management of chilled patties.

Scanga et al. (2000) analyzed both fresh and frozen beef trimmings. The fresh trimmings (n=191) had slightly higher aerobic plate counts than did the frozen (n=111) with counts of 3.3 $\log_{10}$ CFU/g and 2.9 $\log_{10}$ CFU/g, respectively. Total coliform counts were equal with both having a mean of 1.2 $\log_{10}$ CFU/g. *E. coli* had similar counts with a mean of 1.1 $\log_{10}$ CFU/g. *Listeria monocytogenes* was isolated from 3 (1.6%) of the fresh and 7 (6.3%) of the frozen samples, while *Salmonella* was recovered from 6 (3.1%) of the fresh trimmings. Significant aerobic plate count differences were found between processing facilities, as the lowest mean APC was 2.0 $\log_{10}$ CFU/g while the highest was 4.5 $\log_{10}$ CFU/g. However, less than one log difference was found for both coliforms and *E. coli* between all 8 facilities. Core samples were compared to combo-bin purge samples, and it was found that those with a high (>3 $\log_{10}$ CFU/g) mean combo-bin core APC had similar combo-bin purge counts. However, when levels were <3 $\log_{10}$ CFU/g, the purge sample could be as much as 1 log cycle higher. However, correlation coefficients revealed that the APC of core sample to purge samples were highly related (r=0.80) but coliform (r=0.45) and *E. coli* (r=0.43) counts were not strongly related. The authors observed the need for processors to focus efforts upon reducing the microbial load of incoming raw products as well as addressing *Listeria monocytogenes* contamination of raw product.

Mohamood et al. (1992) tested 36 ground beef samples for *Listeria monocytogenes* using a gene probe. *Listeria* spp. was identified in 6 (16.6%) of the samples, of which 4 (11%) were confirmed *L. monocytogenes*. Johnson et al. (1990) sampled 50 beef, 50 pork and 10 lamb roasts for *Listeria* ssp. of which 3 beef and 3 pork roasts were positive. Of the isolates recovered, 5 were *L. monocytogenes*, as well as one each of *L. innocua* and *L. welshimeri*. Forty meat samples (20 beef and 20 poultry) were analyzed by Amoril and Bhunia (1999) for *L. monocytogenes*, with 6 and 7 positive samples, respectively.

These results demonstrate that better hygienic practices are necessary to reduce the contamination of meat products during further processing and at the retail counter. Scanga et al. (2000) as well as Gill et al. (1997) observed a need for reducing the microbial load borne by meat destined for or within the retail market and Amoril and Bhunia (1999) expressed a concern that inadequately cooked raw meat could present a source of infection for *Listeria monocytogenes*. Dixon et al. (1991) was able to demonstrate that improving the sanitation of equipment, reducing cross contamination and utilizing an acid wash system could reduce the contamination of retail meats. The authors proposed the implementation of ante-mortem washing, improving the sanitary procedures during slaughter and carcass breaking as well as utilizing an acid wash system to improve the microbial quality of meat products.

USDA Methods for the Identification of *Listeria monocytogenes*

Many methods have been created to identify and characterize *Listeria monocytogenes*. The USDA/FSIS Microbiology Laboratory Guide Book (1998) established standardized isolation and identification procedures; in October 1999, revisions were released superceded previously published protocols. These changes included the implementation of 'new' technology such such as Polymerase Chain Reaction (PCR) to rapidly confirm negative samples, and DNA fingerprinting.

Testing for *L. monocytogenes* utilizing the USDA/FSIS methodology normally takes 7 to 10 days, depending upon the steps required to recover and identify isolates. The first step involves taking 25 grams of the product being sampled and adding it to 225 ml of University of Vermont Medium (UVM). This is incubated at 30°C for 22 hours. After initial enrichment, 0.1 ml is transferred to 10 ml Fraser broth (UVM + 0.5% Ferric Ammonium Citrate) and incubated at 35°C for 26-28 hours. After 24 hours incubation, all Fraser broth tubes must be struck to Modified Oxford Agar (MOX) that has been supplemented with moxalactam and incubated at 35°C for 24-48 hours. Incubation of the Fraser broth tubes that were struck to MOX must be continued for another 24 hours. After 24 hours, the MOX plates should be examined for distinctive 1-2 mm round colonies surrounded by black zones of esculin hydrolysis. If no colonies are present, the 48-hour Fraser broth should be struck to MOX again, incubated, and examined after 24 hours.

Suspect colonies should be selected, and re-streaked to MOX to ensure purity. Purified colonies should then be struck to horse blood overlay agar, incubated for 19 hours at 35°C and examined for β-hemolysis. It is also necessary that the CAMP test be performed using a commercially available β-lysin disk, or, *S. aureus* and *R. equi* can be streaked in parallel on sheep blood agar, with the presumptive isolate struck horizontally between the two cultures. *Listeria monocytogenes* will give a zone of clearing with the β-lysin disk or with *S. aureus* while *L. innocua* will give no reaction with the disk or with *S. aureus* but it will have a zone of clearing with the *R. equi*. Other required tests include motility testing using either a phase-contrast microscope or motility medium to examine the motility of the culture, and the catalase test using 3% H_2O_2, as *Listeria monocytogenes* is catalase positive. Biochemical testing includes inoculating rhamnose, xylose and mannitol with the suspect isolate, and examining for rhamnose fermentation, as both *L. monocytogenes and L. innocua* are able to utilize rhamnose but not xylose or mannitol, while other species of *Listeria* can ferment either xylose or mannitol but not rhamnose.

In the 1999 revision to the Microbiology Laboratory Guidebook (MLG), several modifications were made to the procedures, most of which were geared toward decreasing the time required to achieve a negative or presumptive positive. The first change involves the addition of streaking or direct plating the UVM enrichment broth to MOX in addition to the continued use of Frasier Broth. The second change allows the use of screening systems, including immunoassay, nucleic acid-based assay or 'other' rapid methods. However,

these rapid systems must be utilized in conjunction with the MOX plate that was struck initially, and only samples that were negative for both the screening procedure and the MOX are considered negative. The third change includes the recommendation that commercially available tests be utilized to examine the biochemical characteristics of isolates. The MLG requires that atypical isolates be confirmed via commercially available RNA or DNA-based tests, and recommends the use of these tests for typical isolates. In April 2002, a “Notice of Change” was published by the USDA identifying the BAX™ PCR system produced by Dupont as an acceptable DNA screening method (Anonymous, 2002). Pulsed-field gel electrophoresis is recommended, but not required, to subtype isolated strains. The original procedure is shown in Figures 1 and 2, and the revised procedure is shown in Figure 3.

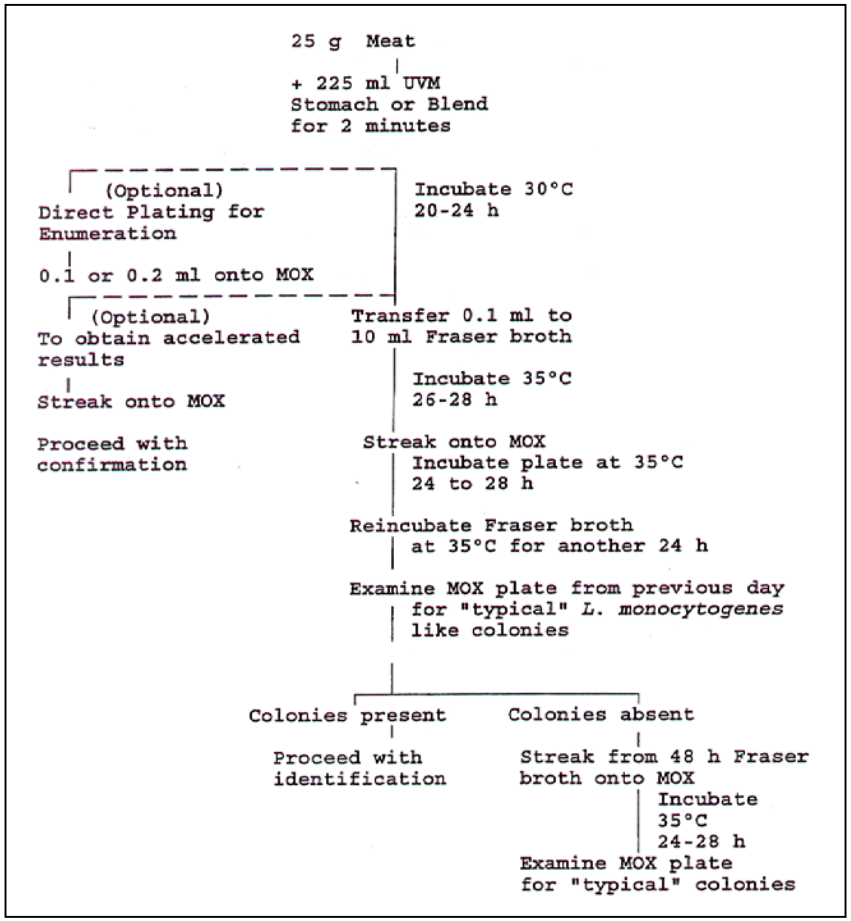

Figure 1. USDA/FSIS MLG *Listeria monocytogenes* Isolation Procedure (1998)

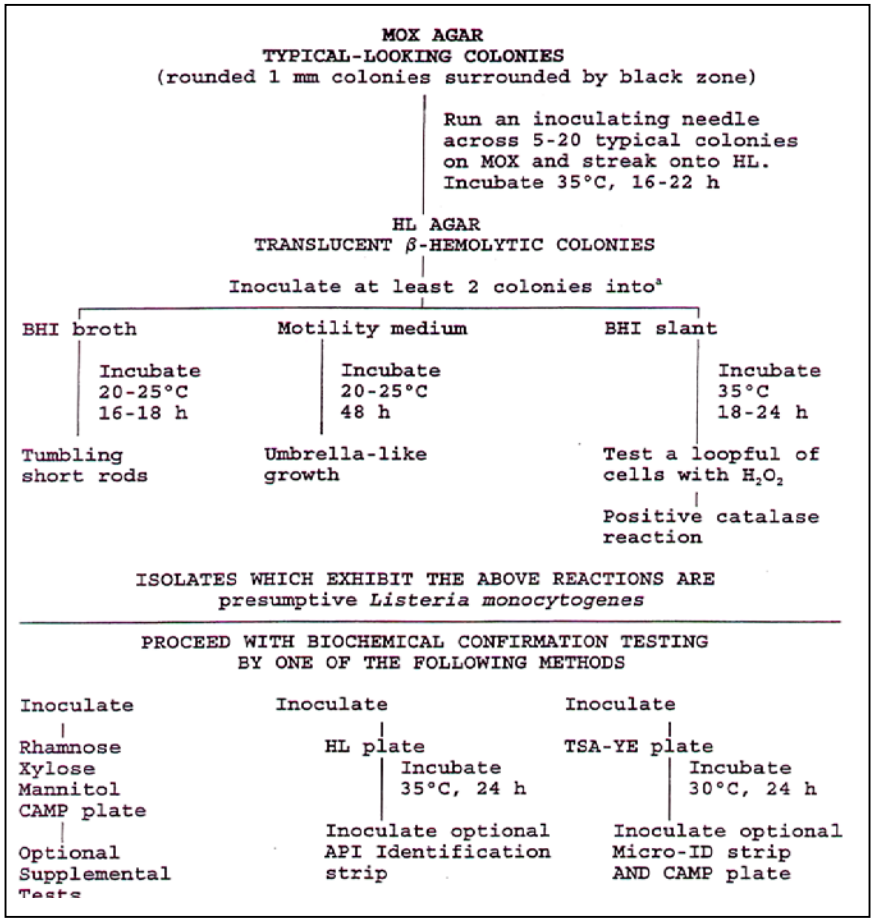

Figure 2. USDA/FSIS MLG *Listeria monocytogenes* Isolation Procedure (1998)

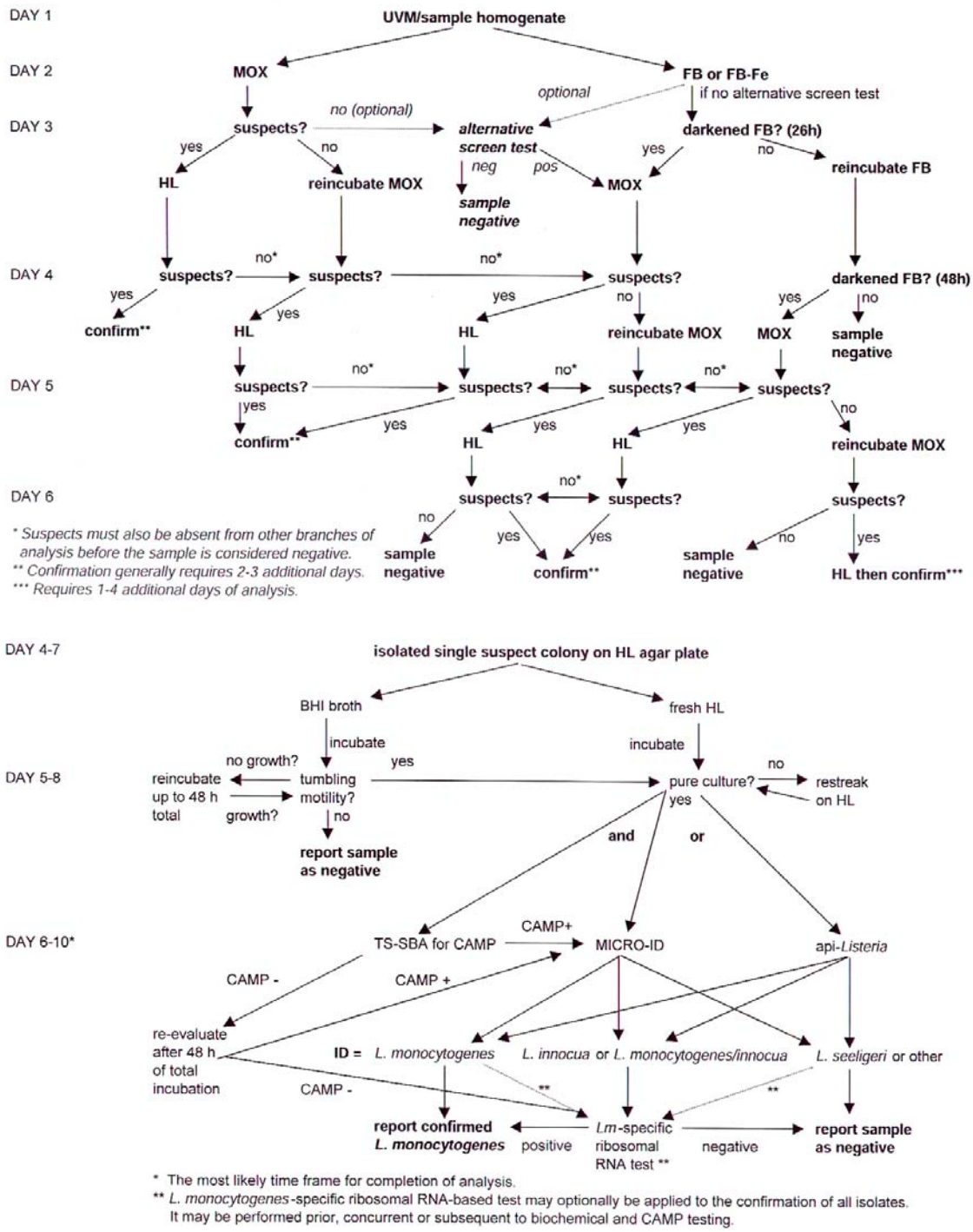

Figure 3. USDA/FSIS MLG *Listeria monocytogenes* Isolation Procedure (Revision 2; 11/08/99)

Identification of *Listeria monocytogenes*

Utilizing the Polymerase Chain Reaction

The 1999 revision to the Microbiology Laboratory Guidebook allowed the use of nucleic acid based analysis of products being examined for *Listeria monocytogenes*. Research has shown that the Polymerase Chain Reaction (PCR) can be a rapid and effective method to determine the presence or absence of an organism in a food product as well as identification or characterization of isolates recovered via traditional isolation methodology. PCR has also been made more 'user friendly' with the advent of commercially available kits such as the BAX™ kit for *Listeria monocytogenes* that provide all the components necessary for cell lysis and PCR amplification.

Research utilizing PCR to identify *Listeria monocytogenes* have included the use of primers specific for the listeriolysin O (*hly* A,), the Dth 18 gene and the invasion associated protein (*iap*) gene (Thomas et al., 1991; Baek et al., 2000) as well as the internalin genes (*inlA, inlB*) (Ericsson et al., 1995). In a study by Baek et al. (2000) primers that amplified a 719 base pair fragment of the listerioysin O gene were used to confirm suspect *Listeria monocytogenes* isolates from a variety of fresh and frozen food products. A total of 1,537 food samples were tested, of which 122 (7.9%) were positive.

Thomas et al. (1991) examined 5 combinations of forward and reverse primers for the *hlyA* gene and identified a 520 base pair (bp) fragment that was most specific to *L. monocytogenes*. This primer set was tested against 47 isolates representing 17 genera of bacteria as well as other *Listeria* spp., with no

false positives. After enrichment, the procedure could detect an initial inoculum as low as 25 CFU of *L. monocytogenes* from milk or meat samples.

Duffy et al. (1999) investigated the use of a polycarbonate membrane that was dipped in the enriched food samples for 15 minutes. The membrane was solubulized to release attached bacteria and the DNA was purified with phenol/chloroform extraction. PCR was then performed using the primers developed by Thomas et al. (1991). When compared with a standard enrichment and plating method, this procedure identified all positive *Listeria monocytogenes* samples, with no false positives, and this procedure provided a result in 30 hours, while the plating method took 96 hours.

The listeriolysin O gene (*hly*A) and invasion-associated protein (iap) were used in a multiplex PCR by Niederhauser et al. (1992) to examine naturally contaminated foods. The *hly*A primers yielded a 234 bp fragment and the *iap* primers yielded a 131 bp fragment. A total of 330 naturally contaminated food samples were tested to compare the multiplex PCR and traditional isolation techniques. The same 20 samples (6.0%) were positively identified by both procedures.

Lawrence and Gilmour, (1994) developed a multiplex PCR that utilized not only the *hlyA* gene, but also two sections of the 16S rRNA, one specific to the genus *Listeria* and the other being highly conserved in all bacteria, regardless of genus. The protocol developed by the authors utilized an enrichment followed by plating on selective media. Suspect colonies were then picked from the plate and subjected to the multiplex PCR. When visualized in an agarose gel, *Listeria*

monocytogenes would have three bands present, *Listeria* spp. would have 2 bands present and a non-*Listeria* spp. would have one band present.

Reverse Transcription-PCR was investigated by Klein and Juneja (1997) to identify samples contaminated with viable *Listeria monocytogenes*. Primers for the *iap, hly* and *prfA* genes were tested, with the *iap* primers giving the best results. When tested in artificially contaminated cooked ground beef, only a 2 hour sample enrichment was required for a positive result in cooked meat. The procedure was able to detect an initial inoculum level as low as 3 CFU/g. The procedure, which included amplicon detection by Southern hybridization with digoxigenin labeled gene probes could be completed in approximately 54 hours.

Colorimetric detection using a PCR-ELISA procedure has also been used to rapidly detect *Listeria monocytogenes*. Scheu et al (1999) utilized the *mpl* (metalloprotease) gene. A biotinylated capture probe was bound to a streptavidin-coated microtiter plate and the hybridization was performed with the PCR product. Digoxigenin-labeled hybridization products were identified with horseradish peroxidase conjugates. Diluted antiDIG-POD was added, the plate was incubated and then rinsed with a wash buffer that contained a blocking agent. A chromogenic substrate solution was then added and the plate was incubated. After the reaction was stopped, the absorbance was measured at 450 nm. A total of 103 strains of *Listeria monocytogenes* as well as 73 other *Listeria* and non-*Listeria* strains were tested, with no false positives or negatives observed. These were similar to the results obtained by Wang and Hong (1999), in which the *iap* gene was utilized and alkaline phosphatase-conjugated

flourescein was added to the plate and the absorbance was determined at 405 nm. The authors also investigated the used of the procedure on artificially contaminated milk and fish, and identified a detection limit of 20 CFU/ml for the milk and 1-2 CFU/g for the fish without the need of an enrichment step.

While a variety of reliable PCR methods have been developed for use in analyzing food products, Qualicon has created a commercially available BAX™ kit for the detection of *Listeria monocytogenes*. This procedure includes both an initial and a secondary enrichment be performed prior to the DNA extraction and PCR analysis being performed. Stewart and Gendel (1998) investigated the specificity and sensitivity of the kit. The authors found that the system was specific for *L. monocytogenes* and gave no reaction with other *Listeria* species or genera tested, and the detection level of the assay was determined to be 10^5-10^6 CFU/ml. Norton et al., (2000) compared the BAX™ kit to conventional isolation procedures as well as confirmation using either an API test kit or primers targeting the *hlyA* gene. A total of 89 samples were positive with the conventional isolation procedure and 80 were positive with the BAX™ procedure. However, the 9 negative BAX™ samples from which isolates were obtained using the traditional method were positive with the BAX™ system when the pure culture was tested. This led the authors to believe that the false-negative results were the result of low *Listeria* numbers after the initial enrichment step, as they only used the initial enrichment broth, not the secondary enrichment for the analysis, and they theorized that the two step enrichment procedure required by Qualicon would increase the sensitivity of the procedure.

The BAX™ procedure has been improved with the development of a real time PCR assay. The amplification of the target DNA generates a fluorescent signal. This signal is detected by the BAX fluorometer, and a result of positive or negative is determined based on the fluorescence of the amplified DNA. The BAX™ system was further given a boost when the 'stamp of approval' was given by the USDA when use of the BAX™ system was included in the Microbiology Laboratory Guidebook on April 29, 2002 (Anonymous, 2002).

Genetic Characterization of *Listeria monocytogenes* by Pulsed-Field Gel Electrophoresis

Genetic characterization of *Listeria monocytogenes* can be performed utilizing a variety of restriction enzymes. Restriction endonuclease analysis (REA) and pulsed-field gel electrophoresis (PFGE) have been utilized to characterize strains of *Listeria monocytogenes* isolated from outbreaks as well as the production environment. While both REA and PFGE utilize restriction enzymes to cleave the DNA, REA typically utilizes restriction enzymes, such as *Hind*III, *Pst*I, *Bam*HI or *Eco*RI and the resulting fragments are separated with a traditional horizontal electrophoresis chamber (Nocera et al., 1990). While REA is useful for separating small DNA fragments (<50 Kb), REA will not separate DNA fragments that are larger than 50 Kb. To separate and visualize these bands, PFGE is required.

PFGE will typically use a restriction enzyme such as *Apa*I, *Asc*I, *Not*I or *Sma*I, which has a large target sequence and occurs less frequently in a

genome, therefore producing large DNA fragments. These fragments are separated using a contour-clamped homogeneous electric field (CHEF) unit (Buchrieser et al., 1992; Brosch et al., 1994). The unit functions by continuously switching the direction of the electrical field. This switching results in the DNA 'snaking' it's way through the agarose gel, and the smaller fragments will travel further down the gel than the larger ones, resulting in identifiable separate bands of DNA being present. While both procedures are useful in typing *Listeria monocytogenes,* the literature indicates that genomic fingerprinting utilizing PFGE is a very discriminating and reproducible method (Proctor et al., 1995).

Howard et al. (1992) investigated the use of PFGE to differentiate *Listeria monocytogenes* from the other species of *Listeria*. The author's research identified *Apa*I, *Asc*I, *Not*I and *Sma*I as the most useful in separating large sized (>50 Kb) DNA fragments. Of these four enzymes, *Asc*I was found to give the best results, as it resulted in more discernable fragments than *Not*I, while *Apa*I and *Sma*I were found to give some fragments that were the result of partial digestion. The authors research also identified a strain of *L. monocytogenes* that had been isolated from a patient was genetically different from the isolate recovered from cheese associated with that illness, although other typing methods had identified them as the same strain. Similar results were observed by Buchrieser et al. (1992), as strains found indistinguishable by phage typing and electrophoretic typing differed upon evaluation by PFGE.

PFGE has also been used in an attempt to correlate DNA patterns with serovar. Brosch et al., (1994) examined 176 strains of *L. monocytogenes* with

*Asc*I and *Apa*I, and identified 63 and 72 unique restriction endonuclease profiles (REDP), respectively. The authors also examined 22 other *Listeria* strains and identified 18 *Asc*I and 19 *Apa*I profiles. Analysis of these profiles resulted in two genomic divisions being identified, of which division I contained serovar's 1/2a, 3a, 3c and 1/2c and division II was found to contain 1/2b, 3b, 4b, 4d, and 4e. One strain of *L. monocytogenes*, ATCC 19116, could be digested with *Asc*I, but was not restricted by *Apa*I. A similar observation was made by Danielsson-Tham et al., (1993), as they were unable to digest some strains of *Listeria innocua* with *Apa*I but were able to digest the DNA utilizing *Sma*I. Neither Brosch et al., nor Danielsson-Tham et al. were able to identify the reason these cultures were not digested by *Apa*I, and neither offered any theories as to the reason certain cultures were indigestible.

Pulsed-field gel electrophoresis has proven useful in evaluating the relatedness of isolates associated with foodborne *Listeria monocytogenes* outbreaks. Proctor et al., (1995) evaluated recalled chocolate milk and cases of Listeriosis to establish a link between the Listeriosis and the milk. The authors also evaluated cultures recovered from several sporadic, but unassociated cases of Listeriosis. The 4 cases associated with the outbreak were found to have the same REDP as isolates recovered from two products produced in the implicated dairy, as well as isolates from an environmental sample of the facility. However, one of the sporadic cases was found to have a 90% similarity to the isolates recovered from the outbreak.

Pereira et al. (1994) characterized 21 isolates of *Listeria monocytogenes* of both food and human origin. The isolates had been previously divided into serogroups 1/2 and 4 , and phage-typing separated them into 8 phagovars, while PFGE further divided the isolates into 12 groups. One of the food isolates, as well as several human isolates that had been phage-typed were 'non-typeable'. However, PFGE revealed the food and human isolates to be the same strain. The authors observed that serotyping, phage typing, and DNA analysis should be performed in combination to properly evaluate isolates of *L. monocytogenes*.

While PFGE is a powerful tool in evaluating isolates associated with outbreaks, it can also be used to evaluate the sources of *Listeria* contamination within the processing environment. Autio et al., (1999) investigated the incidence of *L. monocytogenes* at various production stages of cold smoked salmon and performed PFGE utilizing *Asc*I and *Sma*I. After digestion, the restriction patterns were grouped together into 'pulsotypes' . A total of 9 pulsotypes were identified from the production environment, equipment, and finished product. However, pulsotype I was recovered most frequently (201 of 303 isolates), as this REDP was recovered from the skinning, brining, smoking, slicing and packaging areas. The authors concluded the contamination of the final product was associated with the brining and slicing procedures. Based on these results, the processing plant implemented hot steam, hot air, and hot water processes to control *Listeria monocytogenes*, and subsequently recovered no *Listeria monocytogenes* from 94 samples collected in a 5 month follow up.

In another PFGE study, *Listeria monocytogenes* was isolated and characterized from an ice cream production facility over a 7-year period of time. *Asc*I, *Apa*I and *Sma*I were used to evaluate the recovered isolates by PFGE. A total of 12 PFGE pattern types were identified, with type II being the dominate isolate (26 of 41 isolates). While other isolates of differing patterns were only recovered sporadically, type II was recovered repeatedly over the 7-year sampling period. The authors theorized that the majority of the sporadic isolates recovered were mutants of the type II isolate, as they only differed by one or two bands. The authors also stated that *Asc*I resulted in the best PFGE profile for visual comparison, with more discriminatory patterns than those obtained by *Apa*I or *Sma*I (Miettinen et al., 1999).

Senczeck et al., (2000) collected environmental and product samples from a meat processing facility over a 2-year period of time. After digestion with *Apa*I and *Sma*I, 89 isolates of *L. monocytogenes* were grouped into 15 distinct PFGE-types. As has been observed in other research, 2 of the 15 identified strains persisted within the processing environment and contaminated finished product, while a majority of the isolates were only recovered a single time. Daupin et al. (2001) observed similar results in a fish processing plant, as digestion of DNA recovered from *L. monocytogenes* with *Sma*I and *Apa*I revealed the persistence of a single strain within the environment that was recovered during production, after sanitation, and from the finished product.

Pulsed-field gel electrophoresis has proven very useful in subtyping strains of *Listeria monocytogenes* related to both food outbreaks and the

persistence of this organism within the food processing environment. However, PFGE has traditionally taken several days for plug preparation and endonuclease restriction of the prepared plugs, as well as another 20 to 24 hours for the gel electrophoresis. A standardized protocol developed for the PulseNet DNA Fingerprint Database program has reduced the preparation, digestion and electrophoresis time to 30 hours. The procedure includes the use of *Asc*I and *Apa*I for the restriction digestion and the use of *L.monocytogenes* H2446 as the control strain serves as the DNA fragment size standard. The importance of this procedure lies in the time saved over other published methods that may take 4 to 5 days as well as providing a standardized protocol for participating PulseNet laboratories (Graves and Swaminathan, 2001).

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Chapter 2

INCIDENCE OF LISTERIA SPECIES IN CATTLE HOLDING FACILITIES, FECES, HIDES AND FINISHED BEEF CARCASSES

Abstract

This study investigated the incidence of *Listeria* species in environmental samples of automatic watering systems, composite fecal samples from cattle production facilities, direct fecal samples from cattle at both the production facility and at slaughter, the hides of the cattle during the slaughter process as well as the finished carcasses. Environmental sampling of the abattoir was also performed for *Listeria* species both before and after slaughter. A total of 870 samples were collected, of which 97 (11.1%) were positive for *Listeria* species and 1 (0.11%) was positive for *Listeria monocytogenes*. In the livestock production environment, samples collected included 22 water troughs, 91 composite fecal from the holding pens and 360 individual fecal samples. Of these, 1, 16 and 11 were positive for *Listeria* species, respectively, and 1, a composite fecal sample, was positive for *Listeria monocytogenes*. At the abattoir, 119 individual fecal, 33 hide, 106 carcass and 139 environmental samples were collected, of which 21, 26, 9 and 13 were positive for *Listeria* species, respectively. Pulsed field gel electrophoresis (PFGE) utilizing restriction enzymes *Sma* I and *Asc* I demonstrated that specific strains of *Listeria* have the ability to survive and persist in the production environment, be shed in the feces

of cattle, and ultimately contaminate carcasses. Even though a direct progression of isolates from the feedlot to the carcass may not always be evident, the fact remains that isolates were recovered from the animal production environment were found on finished carcasses, indicating the need for intervention steps for the finished beef carcasses.

Keywords: Listeria; pulsed field gel electrophoresis; *Sma* I; *Asc* I; cattle

1. Introduction

Listeria monocytogenes presents a unique concern for the processor of ready-to-eat (RTE) meats, because it is not considered an adulterant in raw meat products but is considered an adulterant of finished RTE products. However, this organism can present a concern from the standpoint of it entering the processing environment at the time of slaughter (Fenlon et al., 1996) and the subsequent contamination of finished beef carcasses and the finished cuts of meat from those carcasses. Another concern is dissemination through the agriculture production environment. An example of this dissemination would be if this organism was being shed in animal feces and the manure was then used as fertilizer. Vegetables could become contaminated with *Listeria monocytogenes* (Van Renterghen et al., 1991) and cause illness that could potentially be traced back to the production facility (Schlech et al., 1983).

Husu (1990a) investigated 249 dairy herds for shedding of *Listeria* spp. in feces, and on average, 2.12% of the animals in any given herd were shedding *Listeria*. In a second study, Husu et al. (1990b) found that 6.8% of the fecal samples were positive for *L. monocytogenes* and 5.1% were positive for *L. innocua*. This study also found that environmental factors such as feed ingredients and facilities can be contribute to the persistence of *Listeria* within the animal production environment.

The purpose of this study was to isolate *Listeria* spp. from fecal samples collected at the Oklahoma State University Willard Sparks Research Facility from both the animal production environment in the form of composite samples and

directly from the animals as individual fecal grab samples. Samples were also collected from animals at the time of slaughter at the Oklahoma State University Food and Agriculture Products Center abattoir. These samples included collection of fecal samples from the individual animal as well as swabbing the hides and finished beef carcasses.

2. Materials and Methods

Water and fecal samples were collected at the Willard Sparks Research Facility at Oklahoma State University while fecal, hide and carcass swabs were collected in the abattoir of the Food and Agricultual Products Center. Water was collected in sterile 250 ml oak ridge bottles from water troughs at the Willard Sparks Research Facility, and held on ice until transported back to the laboratory. Once transported back to the laboratory, the samples were centrifuged at 9715 x g for 10 minutes to pellet any cells in the water. The water was decanted, and the 'pellet' was resuspended with the enrichment media, and enrichment procedures were begun.

Composite fecal samples were collected from 10 animal droppings within a pen using a sterile tongue depressor, and placed in a Whirl-Pak bag. Individual samples were collected directly from the animal as a 'fecal grab' using a sterile 1.5 inch by 3 inch Whirl-pak sponge to swab or physically remove the sample from the rectum of the animal, either while held in a working chute, or immediately after stunning, but prior to bleeding. Hide swabs were collected from animals after the hocks had been removed, but prior to the hide on the belly

being opened up for removal. Whirl-pak sponges were rehydrated with 0.1% Buffered Peptone Water (Difco, Franklin Lakes, NJ), and as much of the hide as was reasonably possible was swabbed. However, priority was given to areas in which initial incisions were made, such as the centerline of the animal.

Carcass swabs were collected using sterile Whirl-pak sponges rehydrated with approximately 15 ml of buffered peptone water (Difco). An individual sponge was used for each half of the carcass, with both sponges being combined for enrichment. Swabs were collected from the rump, centerline and brisket areas immediately after final trimming and prior to the carcass being washed. Environmental samples were collected with sterile Whirl-pak sponges rehydrated with approximately 15 ml of Neutralizing Buffer (Difco). Environmental samples included drains, the hock cutter, and the carcass saw. After collection, the abattoir samples were immediately transported to the laboratory, and enrichment procedures were begun.

2.1 Detection of Listeria monocytogenes

Isolation protocols were obtained from the USDA-FSIS Microbiology Laboratory Guidebook (USDA, 1998). Water samples, fecal samples, carcass swabs, hide swabs, and environmental samples were enriched in University of Vermont Medium (UVM) broth (Difco) for 24 hours at 30°C. After 24 hours, 1 ml of the initial enrichment was transferred to 9 ml of Fraser broth (UVM with 0.5% Ferric ammonium citrate) and incubated for another 24 hours at 30°C. Black tubes were considered a presumptive positive and were subsequently streaked onto Modified Oxford (MOX) Agar (Difco) and incubated for 48 to 72 hours at

30°C. Isolates exhibiting 'typical' colony morphology (white colony with indented center and esculin hydrolysis evident in agar) were restreaked to MOX agar to purify. Individual isolated colonies were then inoculated into Brain Heart Infusion (BHI) broth for further biochemical testing.

Typical *Listeria* colonies were further examined for catalase production, hemolysin production on 5% Sheep Blood Agar plates (Fisher Scientific, Houston, TX); CAMP test utilizing the ß-lysin disk (Remel, Lenexa, KS), and carbohydrate fermentation patterns on xylose, rhamnose, and mannitol. Isolates from MOX agar found to be Gram-positive, catalase-positive, capable of fermenting rhamnose and exhibiting hemolysis were considered to be potentially *Listeria monocytogenes*. Isolates that exhibited typical morphology but did not produce hemolysin were also considered potential *Listeria monocytogenes* because of possible weak hemolysis reactions. Isolates that produced hemolysin and fermented xylose were considered to be *Listeria ivanovii*. Isolates were confirmed as *Listeria monocytogenes* utilizing the BAX™ PCR kit for *Listeria monocytogenes* (Qualicon, Wilmington, DE). Isolates were stored as frozen stocks for further analysis by centrifuging 10 ml of cells grown overnight in Brain Heart Infusion Broth (Difco) at 7649 x g for 10 minutes. The pellet was resuspended with 2 ml BHI broth supplemented with 10% glycerol and frozen at -75°C. When enough isolates were recovered, DNA fingerprinting with *Asc* I and *Sma* I was conducted to determine the relatedness of recovered isolates.

2.2. PCR Analysis of Isolates

Polymerase chain reaction (PCR) analysis utilizing the BAX™ kit was performed with the only modification of the procedure being the use of a 24 hour culture grown in BHI broth, whereas the BAX™ PCR kit is designed for use in association with an enrichment procedure using MOPS-BLEP. The BAX™ kit comes with its own protease and buffer, of which 62.5 µl of protease solution is added to 5 ml of buffer solution. After mixing, 200 µl of lysis solution was transferred to sterile PCR tubes, and 5 µl of the cell culture was added to the lysis buffer and gently mixed. The solution was heated at 55°C for 60 minutes then at 95°C for 10 minutes to complete the lysis and inactivate the protease. The tubes were then cooled to 4°C prior to use. After completion of the lysis procedures, the DNA lysate can be stored for 2 weeks at 4°C.

The next step in performing the BAX PCR was the addition of 50 µl of lysate to the PCR tubes. The PCR tubes are supplied with a pellet that contains all the necessary ingredients for performing the PCR. After the pellet had dissolved the tubes were loaded into a DNA Engine thermocycler (MJ Research, Reno, NV) and the PCR was performed utilizing the program in Table 1.

Step	Temperature	Time (Minutes)
1	90°C	0:01
2	93°C	2:10
3	94°C	0:25
4	69°C	3:10
5	Repeat step 3 & 4 37 times	
6	4°C	Indefinite

Table 1: Program for BAX PCR Analysis

After completion of the PCR, 20 µl of the PCR product was loaded into a 2% agarose gel at and run at 100V for 60 minutes. The gel was stained utilizing 0.01 mg/ml concentration of ethidium bromide and the PCR product was visualized using a Gel Doc 1000 (Bio-Rad, Hercules, CA).

2.3 Pulsed Field Gel Electrophoresis

Pulsed field gel electrophoresis procedure was done according to Graves and Swaminathan (2001). Cultures were spread on BHI agar and incubated at 30°C overnight to obtain a lawn. The cells were harvested from the BHI agar by adding 1 ml of $T_{10}E_1$ (10mM Tris; 1 mM EDTA) buffer and using a 'hockey stick' or sterile cotton swab to suspend the cells. This cell suspension was pipetted from the plate, and the total volume was adjusted to 3 ml using $T_{10}E_1$. The A_{610nm} was then adjusted to 1.3 using a Spectronic 20 spectrophotometer (Thermo Electron Corporation, West Palm Beach, FL). After adjusting the OD, 240 µl of the cell suspension was transferred to a microcentrifuge tube, and 60 µl of a 10-mg/ml lysozyme solution was added and mixed by gently aspirating the solution. The lysozyme/cell suspension was then incubated at 37°C for 10 minutes.

During the incubation period, 10 ml of a 1.2% Seakem Gold Agarose suspension was melted in sterile deionized water in a microwave and tempered to 56°C. After the cell suspension finished incubating, 267 µl of the agarose, 3 µl of a 10 mg/ml lysozyme solution and 30 µl of pre-warmed (56°C) 10% SDS solution were combined for each sample to be tested, mixed with the lysozyme-treated cell suspension by aspiration several times, and dispensed into disposable 10-well plug molds (Bio-Rad). Each well was filled with 45 µl of the

melted agarose cell suspension, and refrigerated at 4°C for 10 minutes to solidify.

Cell lysis solution (50 mM Tris, pH 8.0; 50 mM EDTA, pH 8.0, 1% Sarcosyl) was prepared by combining 25 ml of 1M Tris-HCL (pH 8.0), 50 ml of 0.5 M 2Na EDTA (pH 8.0) and 50 ml of a 10% sarcosyl solution, and volumetrically adjusting the volume to 500 ml with ultra pure water. Each plug mold was emptied into a sterile 50 ml centrifuge tube, to which 4 ml of lysis solution and 30 µl of proteinase K (20 mg/ml) had been previously added. The plugs were then incubated with shaking at 56°C for 2 hours. After incubation, the cells were washed twice with 15 ml of pre-heated (50°C) sterile water for 10 minutes with shaking. The plugs were then washed twice with 15 ml of preheated (50°C) $T_{10}E_1$ with shaking at 50°C and twice with room temperature $T_{10}E_1$ solution. The plugs could then be used immediately, or stored at 4°C until needed.

2.4 Restriction with Sma I.

The plugs selected for digestion were first washed in 150 µl of 1X *Sma* I (Fisher Scientific) buffer solution supplemented with 1x-strength Bovine Serum Albumin for 10 minutes at 25°C. This was accomplished by placing one prepared plug in a 1 ml microcentrifuge tube and adding the buffer solution. After incubation, the buffer was aspirated from the tube, and 1x buffer containing 5 U of *Sma* (Fisher Scientific, Houston, TX) was added to the tube and incubated for a minimum of 4 hours at 25°C. A 0.5x TBE solution was utilized as the buffer in

the electrophoresis chamber and as the solution in which the agarose was melted.

Digested plugs were loaded into a 15 well gel mold (Bio-Rad) containing 1% Seakem Gold Agarose, and covered with molten agar to prevent the plugs from coming out during the electrophoresis. The agarose gel was loaded into the electrophoresis chamber containing 3000 ml of chilled (15°C) buffer with a flow rate of 0.75 liters per minute. The ramp times for *Listeria monocytogenes* digested with *Sma* I were obtained from research by Boerlin et al. (1997). The electrophoresis control module was a CHEF DR III (Bio-Rad) set to a ramp time of 1.0 second to 9.0 seconds, a run time of 18 hours and 6.0 V/cm. After completion of electrophoresis, the gel was visualized using ethidium bromide staining (0.01 mg/ml) and a Gel Doc 1000 (Bio-Rad). Analysis was performed using DNA Fingerprinting Plus software (Bio-Rad).

2.5 Restriction with Asc I.

Procedures for digestion with *Asc* I (New England Biolabs, Inc., Beverly, MA) differ from the procedure for *Sma* I in the buffer, ramp times and run times used. The plugs were washed in 150 µL of 1x NEBuffer 4 for 15 minutes. After washing, the buffer was aspirated from the microcentrifuge tube, and replaced with 1x NEBuffer 4 containing 1.25 U of *Asc* I. The tube was incubated for a minimum of 4 hours at 37°C. The agar, chamber buffer and plugs were prepared the same as previously mentioned. The electrophoresis control module was set to a ramp time of 4 seconds to 40 seconds, and a run time of 20 hours at 6.0 V/cm. The gel was visualized as previously mentioned.

3. Results and Discussion

A total of 870 samples were collected and tested for *Listeria* spp. and *Listeria monocytogenes* during a 16-month period between April 12, 1999 and August 17, 2000. These samples were collected from the OSU Willard Sparks Beef Research Facility and the Oklahoma Food and Agriculture Products Research and Technology Center (FAPC). Samples collected from Willard Sparks included water from the animals shared water troughs, composite fecal samples collected from the feedlot environment in which multiple animals were penned, and 'fecal grab' samples collected from individual animals. Samples collected from the FAPC included 'fecal grab' samples, hide swabs, carcass swabs and environmental swabs. A total of 870 samples were collected, of which 97 (11.1%) were identified as *Listeria* spp. and 1 (0.11%) was identified as *Listeria monocytogenes*.

3.1 Listeria *Sampling Results*.

A total of 473 samples were collected at the Willard Sparks Research Facility (Table 2). Water troughs that were sampled each supplied water to two pens. Municipal water was supplied to an 'open' type trough, in which the water filled an open trough that was exposed to the production environment. However, only one *Listeria* spp. isolate was recovered from the 22 (4.5%) samples that were collected. This is considerably lower percentage positive than that found by Husu et al. (1990a), in which they found 54% of the water cups sampled were positive for *Listeria* spp.

Composite fecal samples were collected 91 times, with *Listeria monocytogenes* recovered once (1.1%) and *Listeria* spp. recovered on 16 (17.6%) occasions (Table 5). This is similar to the results found by Siragusa et al. (1993) in which 1.4% of the composite samples collected were positive for *L. monocytogenes* and 20% were positive for *Listeria* spp. However, different results have been found by Husu et al. (1990b) in which 6.8% of the composite fecal samples were positive for *L. monocytogenes* and 5.1% were positive for *L. innocua.* In another study, Husu et al. (1990b) recovered *L. innocua* and *L. monocytogenes* from 21.7% and 7.3% of the composite samples collected, respectively. It is important to note, however, that the samples collected by Husu et al. (1990a) and Husu et al. (1990b) were from dairy farms, not a feedlot environment, thus, different feeding or handling practices could contribute to the observed differences.

Facility Name	**Sample Type**	**# of Samples**	***Listeria* spp.**	***Listeria monocytogenes***
Willard Sparks	Water	22	1	0
	Composite Fecal	91	16	1
	Fecal Grab	360	11	0
FAPC Abattoir	Fecal Grab	119	21	0
	Hide Swab	33	26	0
	Carcass Swab	106	9	0
	Environmental	139	13	0
	Total=	870	97	1

Table 2: Facilities sampled for *Listeria* spp. and *Listeria monocytogenes*

Individual, or 'direct fecal grab', samples were collected from 360 head of cattle being fed for slaughter at the Willard Sparks Research Facility (Table 2). *Listeria* spp. were recovered from 11 (3.1%) of these animals while *L.*

monocytogenes was not recovered from any of the samples. While it was surprising that *L. monocytogenes* was not recovered from the cattle, these data confirm those of Siragusa et al. (1993) who sampled 138 head of cattle and only recovered *L. monocytogenes* once (0.72%).

When cattle were slaughtered at the Food and Agriculture Products Research and Technology Center (FAPC), the cattle to be harvested were received from multiple sources, including local producers as well as the Willard Sparks Research Facility. Fecal samples collected from the FAPC had a higher percentage positive than those from the Willard Sparks Research Facility, as *Listeria* spp. was isolated 21 times from 119 (17.6%) samples. However, as in the samples from Willard Sparks, *L. monocytogenes* was not recovered. The increase in the number of samples positive for *Listeria* spp. could be a result of a variety of factors, including the environment in which the animals had resided prior to transport and the stress related to transport to the facility.

Hide swabs were collected from 33 animals, of which 26 (78.8%) were positive for *Listeria* spp. However, *L. monocytogenes* was not recovered from any hide swabs. The percent positive for *Listeria* species on hides was much greater than that found in the feces. This difference between incidence in feces and on hides is not unique to *Listeria* spp. In research by Elder et al. (2000), examining fecal samples and hides for *E. coli* O157:H7, 27.8% of the hide swabs were positive for *E. coli* O157:H7 while only 10.7% of the direct fecal samples were positive. This phenomenon can lend itself to the observation that the feces

from one animal is contaminating the hide of not only itself, but also those of other animals either in the livestock production facility or during shipping.

After the carcasses were split, but before washing, carcass swabs were collected. A total of 9 out of 106 samples (8.5%) were positive for *Listeria* spp. When compared to the number of hides in which *Listeria* spp. were identified, the importance of hygienic practices are exemplified. Elder et al. (2000) found that while 27.8% of the cattle hides sampled were contaminated with *E. coli* O157:H7, only 1.8% of the carcasses were positive post processing. However, the fact that carcasses can still become contaminated demonstrates the need for further carcass decontamination steps, such as acid washes or steam pasteurization to further reduce carcass contamination. In fact, the USDA-FSIS issued Notice 44-02 (USDA, 2002) that advised facilities of their obligation to reassess their HACCP plans and determine if *E. coli* O157:H7 is a hazard likely to occur, and if identified as such, then appropriate Critical Control Points such as acid washes or steam pasteurization will be required for controlling this hazard. Also, the USDA-FSIS has mandated that monitoring of carcasses for the presence of fecal contamination or ingesta is a required CCP.

Environmental samples were collected from the abattoir both before and after slaughter to evaluate the environment for the presence of *Listeria* spp. A total of 139 samples were collected with 1 (0.72%) sample positive pre-slaughter and 12 (8.6%) positive after production was complete but prior to sanitation beginning. Environmental samples included drains, the hock cutter, the carcass saw, wizard knifes, inedible barrels and drum dolly wheels. Of the areas that

were positive, *Listeria* spp. was recovered once from a drain prior to the start of slaughter while the remaining positives were recovered from the hock cutter (3 times) and various drains (9 times) after production was complete.

Molecular characterization was performed on the environmental samples, and a majority of the isolates recovered from environmental samples did not closely match (>90% similarity) the fingerprint of isolates recovered from the fecal, hide or carcass swabs. These results are not easily explained, and several different scenarios could exist for the results that were obtained. The first possibility is that these isolates were present prior to slaughter and were not recovered during the pre-production sampling. It is also possible that these strains were in low enough numbers in the feces or on the hide in relation to the other strains of *Listeria* that they were not recovered from the hide or fecal samples. These strains could also have been easily displaced from the hide or feces of the animal, thereby allowing their recovery from the environment.

3.2 *Molecular Characterization*.

Isolates recovered from all facilities that were rhamnose-positive were tested by polymerase chain reaction (PCR) to determine if the isolate was *L. monocytogenes.* A total of 97 isolates were tested, of which 1 was confirmed *L. monocytogenes*. This sample was a composite fecal sample collected from a set of pens at the Willard Sparks Research Facility that housed cattle only once during the time samples were collected. These pens were located on the North side of the facility barn, and designated as ‘N MP 18’. Due to the large number of negative samples (i.e., few positive samples), DNA fingerprinting was

performed on all samples for which *Listeria* spp. were obtained. After grouping the isolates by PFGE analysis, one PCR sample from each pulsotype grouping was re-run on an agarose gel. These results are shown in Figures 4, 5, and 6.

All samples were DNA fingerprinted using the procedure of Graves and Swaminathan (2001). The only modifications to the procedures provided by Graves and Swaminathan was the use of *Sma* I as a restriction enzyme for use in PFGE. This enzyme was used because *Apa* I resulted in incomplete digestion of the DNA of many of our isolates, a phenomenon also observed by Brosch et al. (1994) and Danielsson-Tham et al. (1993). Digestion with *Asc* I resulted in 23 different patterns while *Sma* I gave 29 different patterns. After analysis with Bio-Rad's DNA Fingerprinting Plus software, each different pattern was assigned a letter, ranging from A to W for *Asc* I and A to CC for *Sma* I. The individual sample and the 'pulsotype' for *Asc* I and *Sma* I are shown in Tables 6A, 6B, and 6C.

Groupings were determined by evaluating the results of the Fingerprinting Plus software, which has a built in function that will identify the visible bands on the gel image, then automatically group the fingerprints based on the relatedness of the DNA bands present in the gel image. Any fingerprints showing greater than 90% similarity were grouped together. A total of 39 different PFGE groupings were identified among the 97 strains after analysis and sorting. Grouping of *Asc* I isolates was simplified by the low frequency of cuts, as well as the clarity of the digested DNA fragments. *Sma* I, on the other hand, frequently

resulted in 18 to 20 bands, that had a tendency to contain a number of relatively 'unclear' fragments when compared to the *Asc* I fingerprints.

3.3 *Groupings by Pulsotype.*

A total of 39 pulsotype groupings were identified when the DNA fingerprints for each restriction enzyme were combined (Table 3A, 3B & 3C). The most frequently recovered isolated was identified as pulsotype FS, as this particular strain of *Listeria* was recovered 33 times over the 16 month sampling period. This strain demonstrated the ability to survive in the production environment, and was recovered from both composite and direct fecal samples at Willard Sparks as well as from fecal and hide swabs from cattle being slaughtered at the FAPC. However, as often as this organism was recovered from the animal (20 fecal, 11 hide samples), it was never recovered from a carcass swab.

Pulsotypes NE, NF, NG and NH fell outside the 90% standard established for the grouping of pulsotypes generated by both enzymes to be the same organism. However, these similar isolates had an 85% overall relatedness with the *Sma* I enzyme. Therefore, while they may not be identical, the isolates appear to be highly related. These groupings (NE, NF, NG and NH) account for 11 of the 97 (11.3%) isolates recovered. These 11 isolates were all recovered from hides (9 isolates) or fecal (3 isolates) samples, with no positive carcass samples for this strain of *Listeria*.

On April 25 to 27, 2000, samples were collected from animals slaughtered in the FAPC abattoir that had been housed at the Willard Sparks Research Facility. A total of six isolates were recovered from the carcass swabs that

matched composite fecal samples collected at Willard Sparks on June 9, 1999 and June 15, 1999 as well as a carcass swab collected on October 14, 1999. These isolates were all identified as pulsotype grouping WZ. Similar results were observed from samples collected on April 5, 2000. Two fecal samples and one carcass sample were positive, and DNA fingerprinting revealed they had the AD pulsotype grouping. These sampling results subsequently led to the inclusion of hide sampling in an attempt to link the hide as a source of contamination, as these carcasses were positive, but the fecal samples collected from them did not result in this organism being recovered. These results further suggest that *Listeria* spp. could maintain a population in an animal pen environment (11 months or more) even when animals were not kept within that environment the entire time.

Subsequent samples collected in July and August of 2000 did result in the same pulsotype grouping (ES) being recovered from fecal, hide and carcass swabs. A total of 8 isolates were recovered, of which 5 were fecal, 2 were hide and 1 was carcass. The first isolate was recovered from a hide sample on July 5, 2000. The second isolate was recovered from a fecal sample on August 9, 2000, and the remaining isolates were recovered on August 10, 2000. This *Listeria* isolate was recovered from fecal samples of animals 9, 10, 11 and 12, as well as a carcass sample from animal number 12 and a hide sample from animal number 13. However, pulsotype groupings FS and NG were also recovered from the feces, and FS, NG, NF, LW and GS were recovered from the hides.

Pulsotype NG was recovered from the hide of animal number 12, but pulsotype-grouping ES was recovered from both the feces and carcass.

The results obtained from the fecal, hide and carcass sampling are somewhat varied an unpredictable, as it would be anticipated that isolates recovered from the hide and/or fecal swabs would have a high probability of matching those recovered from carcasses. However, this was not necessarily the case. On July 5 and 6, 2000, a total of 12 animals (carcass, fecal and hide) were sampled. Of those samples, 4 fecal samples were positive for *Listeria*, with the pulsotype groupings of strains isolated being FH, FT and FS. All 12 hide samples were positive for *Listeria* with isolates recovered from pulsotype groupings FS, HU, NF, ES, NH, and AS. *Listeria* was only recovered from one carcass swab, and it had a pulsotype grouping of BO, and while there is some relatedness shared between some of the hide and fecal samples, many of the recovered carcass isolates had little relatedness. It is also important to note that pulsotype FS was recovered from the pen in which these animals were housed on the day of slaughter.

When the sampling began in the processing environment, it was anticipated that isolates would be recovered from the processing environment that matched those recovered from the cattle being slaughtered. However, while two of the isolates recovered were pulsotype group FS, the other 11 isolates recovered from the abattoir were not previously recovered from Willard Sparks or from sampled cattle. Also, *Listeria* were not recovered from any pre-slaughter environmental samples, but were recovered during post-slaughter environmental

sampling. Therefore, these isolates either were present and not recovered during the pre-slaughter sampling due to an unidentified niche, or they were present on the animals being sampled, and were not recovered through the isolation process from the feces or carcasses, but were recovered from the environment.

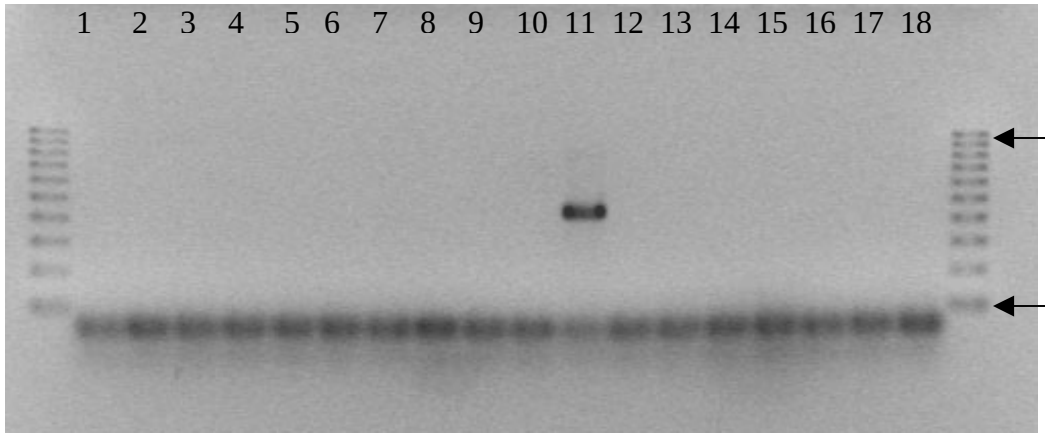
A number of other isolates were recovered from fecal and hide swabs, from both Willard Sparks and the FAPC that were not subsequently recovered any other times (sporadic isolates). These results were not particularly surprising, as it would be anticipated that some strains would enter the production environment for a short time, but not survive (i.e. transient strains), while other strains of *Listeria* would be able to survive within the environment and become persistent strains.

4. Conclusions

A variety of strains of *Listeria* were isolated from the cattle at the Willard Sparks Research Facility as well as the Food and Agriculture Products Center. It has been demonstrated that, while specific strains have the ability to survive and persist in the production environment, or be shed in the feces of cattle and contaminate the hides of cattle, these strains may not pose a serious threat to the contamination of the finished carcass, as 8 of the 9 carcass isolates recovered had a pulsotype that had only been recovered from the production facility on 3 occasions. It has also 'muddied the water' as to the true source of carcass contamination, as the isolates recovered from a finished carcass may not match the isolates recovered from the feces or hide of the animal being

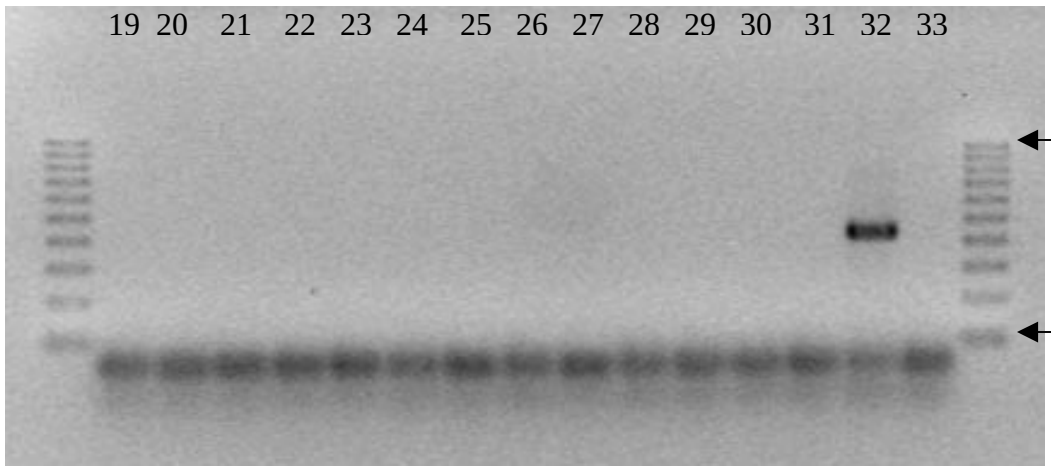
slaughtered or a sufficient number of ‘upstream’ isolates not recovered to ensure a ‘downstream’ match. It is also highly probable that multiple strains may be present in the feces or hides but only one strain may be recovered during the isolation process. It is possible that the contamination could be a result of cross contamination, from people contacting the hide or fecal material of one animal and transferring it to another animal or carcass or from the animals themselves coming in contact with each other during the transportation and slaughtering process.

It is also important to realize that while a direct progression of a specific strain from the animal production facility to the feces, the hide and ultimately the carcass may not always be evident, the fact remains that if isolates are recovered from both the production environment and the carcass, there is a need to ensure that intervention steps are in place.



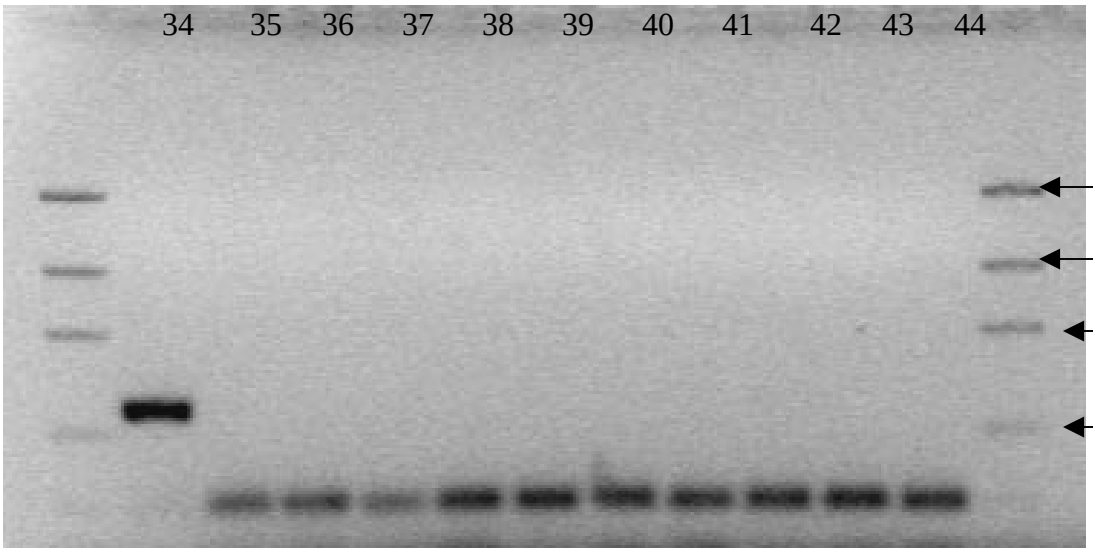
Lane	*Asc* I	*Sma* I	PCR Result
1	F	S	Negative
2	G	V	Negative
3	T	Y	Negative
4	K	E	Negative
5	N	E	Negative
6	W	Z	Negative
7	J	Q	Negative
8	P	K	Negative
9	G	S	Negative
10	F	R	Negative
11	F	AA	Positive
12	F	G	Negative
13	O	S	Negative
14	K	S	Negative
15	V	B	Negative
16	R	C	Negative
17	D	X	Negative
18	C	J	Negative

Figure 4: PCR results for *Listeria monocytogenes*.



Lane	*Asc* I	*Sma* I	PCR Result
19	M	P	Negative
20	A	D	Negative
21	P	M	Negative
22	R	AA	Negative
23	F	H	Negative
24	H	U	Negative
25	F	T	Negative
26	B	O	Negative
27	N	F	Negative
28	E	S	Negative
29	N	H	Negative
30	A	S	Negative
31	E	S	Negative
32	L. monocytogenes	Scott A	Positive
33	L. innocua	ATCC 33090	Negative

Figure 5: PCR results for *Listeria monocytogenes*.



Lane	*Asc* I	*Sma* I	PCR Result
34	*L. monocytogenes*	Scott A	Positive
35	L. innocua	ATCC 33090	Negative
36	Q	E	Negative
37	F	BB	Negative
38	I	S	Negative
39	U	BB	Negative
40	P	L	Negative
41	N	G	Negative
42	N	F	Negative
43	L	W	Negative
44	S	CC	Negative

Figure 6: PCR results for *Listeria monocytogenes*.

Isolate Location	Date	*Asc* I	*Sma* I
FAPC #821 F	4/5/2000	A	D
FAPC #814 F	4/5/2000	A	D
FAPC #2 C	4/5/2000	A	D
FAPC 11 H	7/6/2000	A	S
FAPC 2 C	7/5/2000	B	O
FAPC Holding Pen Drain-post	3/28/2000	C	J
FAPC Evisceration Drain-post	3/28/2000	D	X
FAPC 5 H	7/5/2000	E	S
FAPC 6 F	8/9/2000	E	S
FAPC 9 F	8/10/2000	E	S
FAPC 10 F	8/10/2000	E	S
FAPC 11 F	8/10/2000	E	S
FAPC 12 F	8/10/2000	E	S
FAPC 12 C	8/10/2000	E	S
FAPC 13 H	8/10/2000	E	S
W.S. N MP 18 F	4/16/2000	F	AA
FAPC Carcass Saw Drain-post	7/5/2000	F	BB
W.S. Direct Fecal	11/8/1999	F	G
FAPC 1 F	7/5/2000	F	H
W.S. Direct Fecal	11/8/1999	F	R
W.S. N 1 F	4/12/1999	F	S
W.S. N 3 F	4/12/1999	F	S
W.S. N 1 F	6/15/1999	F	S
W.S. N 21 F	6/15/1999	F	S
W.S. Direct Fecal	11/8/1999	F	S
W.S. Direct Fecal	11/8/1999	F	S
W.S. Direct Fecal	11/8/1999	F	S
W.S. Direct Fecal	11/8/1999	F	S
W.S. Direct Fecal	11/8/1999	F	S
W.S. Direct Fecal	11/8/1999	F	S
W.S. Direct Fecal	11/8/1999	F	S
FAPC #3 F	2/11/2000	F	S
FAPC #4 F	2/11/2000	F	S

F= Fecal, C=Carcass swab, H=Hide swab, W=Water
Pre=Pre-slaughter, Post=Post-slaughter

Table 3A: Sampling Location, PFGE Grouping and Organism Identification

Isolate Location	Date	*Asc* I	*Sma* I
FAPC #3 F	2/15/2000	F	S
FAPC Hock Cutter-post	4/5/2000	F	S
FAPC 1 H	7/5/2000	F	S
FAPC 6 F	7/5/2000	F	S
FAPC 7 F	7/6/2000	F	S
FAPC 7 H	7/6/2000	F	S
FAPC 9 H	7/6/2000	F	S
FAPC 10 H	7/6/2000	F	S
FAPC 12 H	7/6/2000	F	S
FAPC Bleeding drain-post	7/5/2000	F	S
W.S. MP 5 F	7/6/2000	F	S
FAPC 3 F	8/9/2000	F	S
FAPC 7 F	8/10/2000	F	S
FAPC 8 F	8/10/2000	F	S
FAPC 1 H	8/9/2000	F	S
FAPC 3 H	8/9/2000	F	S
FAPC 4 H	8/9/2000	F	S
FAPC 9 H	8/10/2000	F	S
FAPC 11 H	8/10/2000	F	S
FAPC 5 H	8/17/2000	F	S
FAPC 2 F	7/5/2000	F	T
W.S. Direct Fecal	11/8/1999	G	S
FAPC 10 H	8/10/2000	G	S
W.S. N 16 F	5/26/1999	G	V
FAPC 2 H	7/5/2000	H	U
FAPC Evisceration Drain-post	7/5/2000	I	S
W.S. S 5/6 W	8/11/1999	J	Q
W.S. N 30 F	6/9/1999	K	E
W.S. Direct Fecal	11/8/1999	K	S
FAPC 6 H	8/9/2000	L	W
FAPC #3 F	4/5/2000	M	P
W.S. N 32 F	6/9/1999	N	E
FAPC 3 H	7/5/2000	N	F
FAPC 4 H	7/5/2000	N	F

F= Fecal, C=Carcass swab, H=Hide swab, W=Water
Pre=Pre-slaughter, Post=Post-slaughter

Table 3B: Sampling Location, PFGE Grouping and Organism Identification

Isolate Location	Date	*Asc* I	*Sma* I
FAPC 8 H	7/6/2000	N	F
FAPC 5 H	8/9/2000	N	F
FAPC 7 H	8/10/2000	N	F
FAPC 5 F	8/9/2000	N	G
FAPC 2 H	8/9/2000	N	G
FAPC 8 H	8/10/2000	N	G
FAPC 12 H	8/10/2000	N	G
FAPC 6 H	7/5/2000	N	H
FAPC #2 F	2/11/2000	O	S
FAPC Red Angus F	10/7/1999	P	K
W.S. MP 10 F	7/6/2000	P	L
FAPC Holding Pen Drain-post	4/5/2000	P	M
FAPC Hock Cutter Drain-post	7/5/2000	Q	E
FAPC Holding Pen Drain-post	4/27/2000	R	A
FAPC Hock Cutter Drain-post	3/28/2000	R	C
FAPC Evisceration Drain-pre	8/15/2000	S	CC
FAPC Hock cutter Drain-post	8/15/2000	S	CC
W.S. N 19 F	5/26/1999	T	Y
FAPC Evisceration Drain-post	7/6/2000	U	BB
FAPC #815 F	3/28/2000	V	B
W.S. N MP 13 & 14 F	4/16/2000	W	S
W.S. N 23 F	6/9/1999	W	Z
W.S. N 25 F	6/9/1999	W	Z
W.S. N 19 F	6/15/1999	W	Z
FAPC White Face C	10/14/1999	W	Z
FAPC 6 C	4/25/2000	W	Z
FAPC 7 C	4/25/2000	W	Z
FAPC 8 C	4/25/2000	W	Z
FAPC 2 C	4/26/2000	W	Z
FAPC 3 C	4/27/2000	W	Z

F= Fecal, C=Carcass swab, H=Hide swab, W=Water
Pre=Pre-slaughter, Post=Post-slaughter

Table 3C: Sampling Location, PFGE Grouping and Organism Identification

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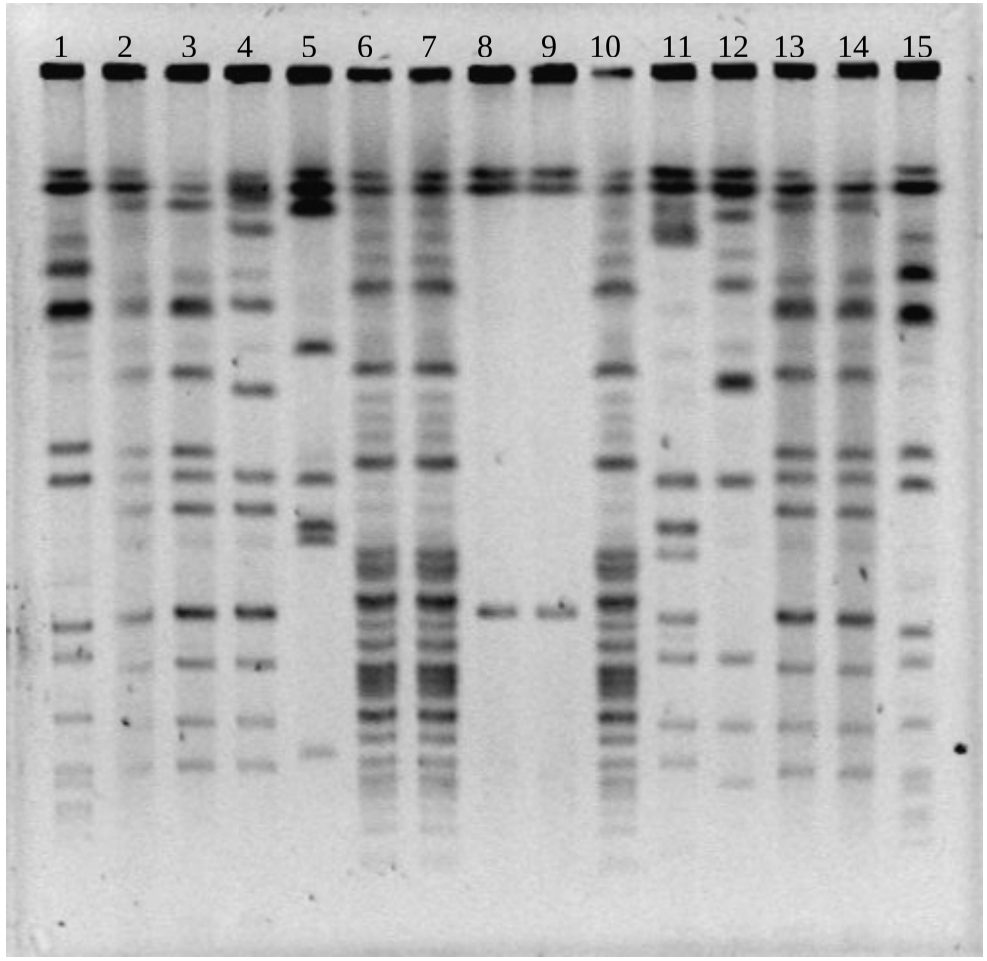
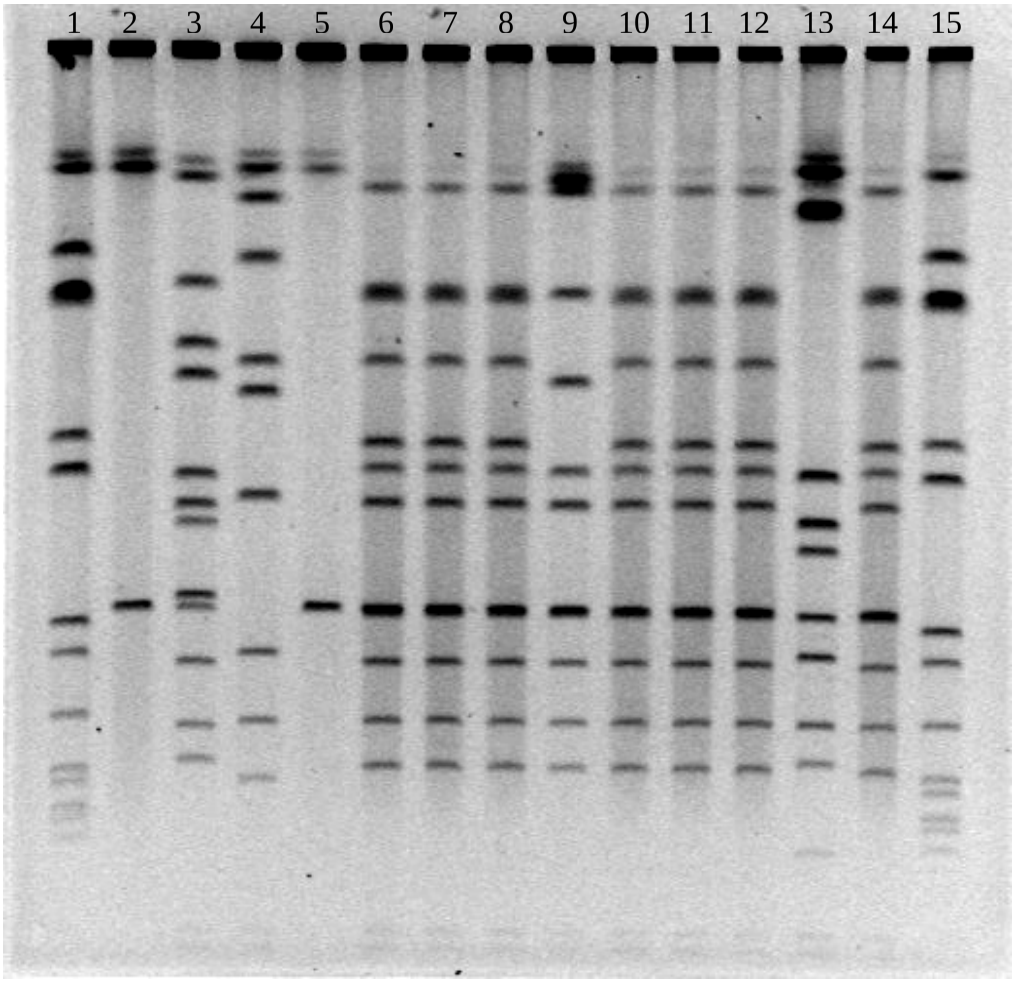
APPENDIX

PULSED FIELD GEL ELECTROPHORESIS IMAGES



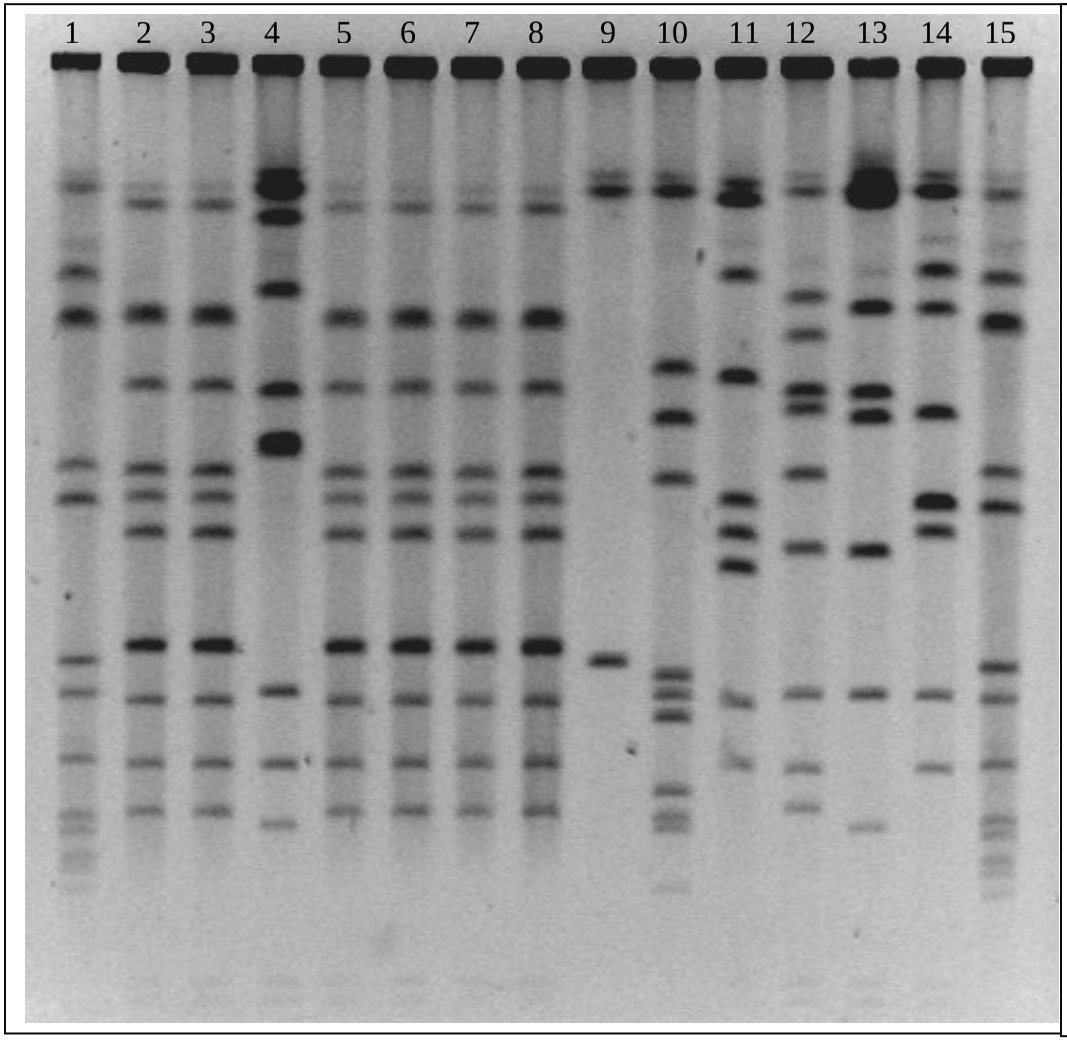
Lane	Source	Date	Group
1	H2446		
2	W.S. N 1 Fecal	4/12/99	F
3	W.S. N 3 Fecal	4/12/99	F
4	W.S. N 16 Fecal	5/26/99	G
5	W.S. N 19 Fecal	5/26/99	T
6	Non-*Listeria* Spp.		
7	Non-*Listeria* Spp.		
8	W.S. N 23 Fecal	6/9/99	W
9	W.S. N 25 Fecal	6/9/99	W
10	Non-*Listeria* Spp.		
11	W.S. N 30 Fecal	6/9/99	K
12	W.S. N 32 Fecal	6/9/99	N
13	W.S. N 1 Fecal	6/15/99	F
14	W.S. N 21 Fecal	6/15/99	F
15	H2446		

Figure 1: Pulsed Field Electrophoresis of *Listeria* spp. digested with *ASC* I; Pulsotypes F, G, T, W, K, N



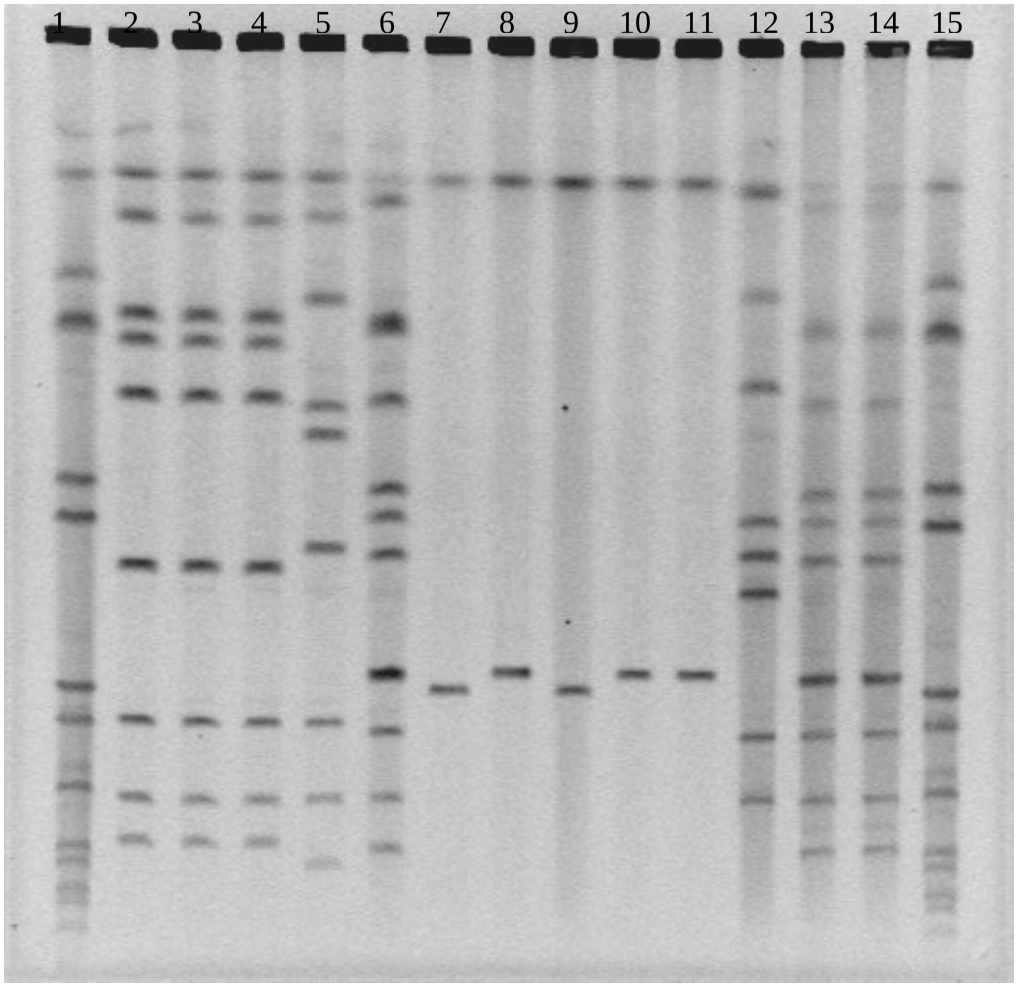
Lane	Source	Date	Group
1	H2446		
2	W.S. N 19 Fecal	6/15/99	W
3	W.S. S 5/6 Water	8/11/99	J
4	FAPC Red Angus Fecal	10/7/99	P
5	FAPC White Face Carcass	10/14/99	W
6	W.S. Direct Fecal	11/8/99	F
7	W.S. Direct Fecal	11/8/99	F
8	W.S. Direct Fecal	11/8/99	F
9	W.S. Direct Fecal	11/8/99	G
10	W.S. Direct Fecal	11/8/99	F
11	W.S. Direct Fecal	11/8/99	F
12	W.S. Direct Fecal	11/8/99	F
13	W.S. Direct Fecal	11/8/99	K
14	W.S. Direct Fecal	11/8/99	F
15	H2446		

Figure 2: Pulsed Field Electrophoresis of *Listeria* spp. digested with *ASC* I; Pulsotypes W, J, P, F, G, K



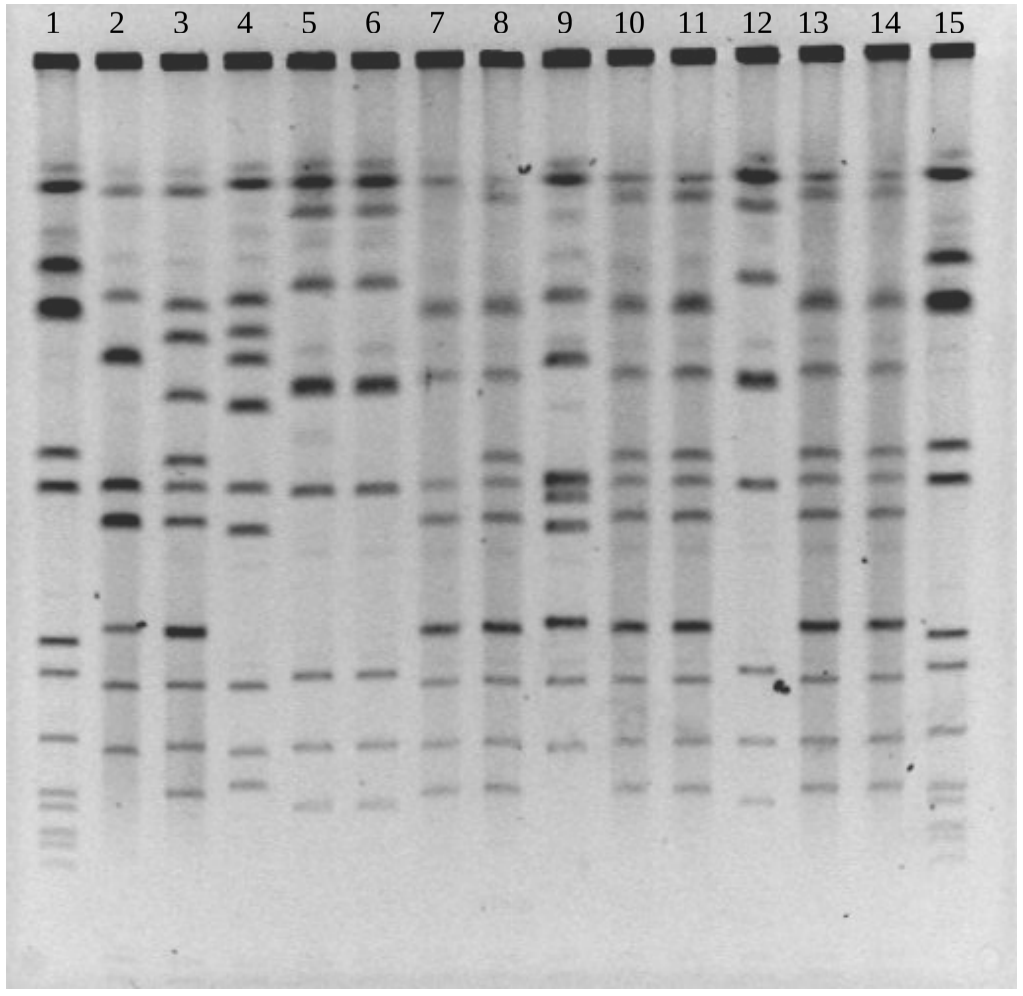
Lane	Source	Date	Group
1	H2446		
2	W.S. Direct Fecal	11/8/99	F
3	W.S. Direct Fecal	11/8/99	F
4	FAPC #2 Fecal	2/11/00	O
5	FAPC #3 Fecal	2/11/00	F
6	FAPC #4 Fecal	2/11/00	F
7	FAPC #3 Fecal	2/15/00	F
8	W.S. N MP 13/14 Fecal	4/16/00	W
9	W.S. N MP 18 Fecal	4/16/00	F
10	FAPC #815 Fecal	3/28/00	V
11	FAPC Hock Cutter Drain	3/28/00	R
12	FAPC Evisceration Drain	3/28/00	D
13	FAPC Holding Pen Drain	3/28/00	C
14	FAPC #3Fecal	4/5/00	M
15	H2446		

Figure 3: Pulsed Field Electrophoresis of *Listeria* spp. digested with *ASC* I; Pulsotypes F, O, W, V, R, D, C, M



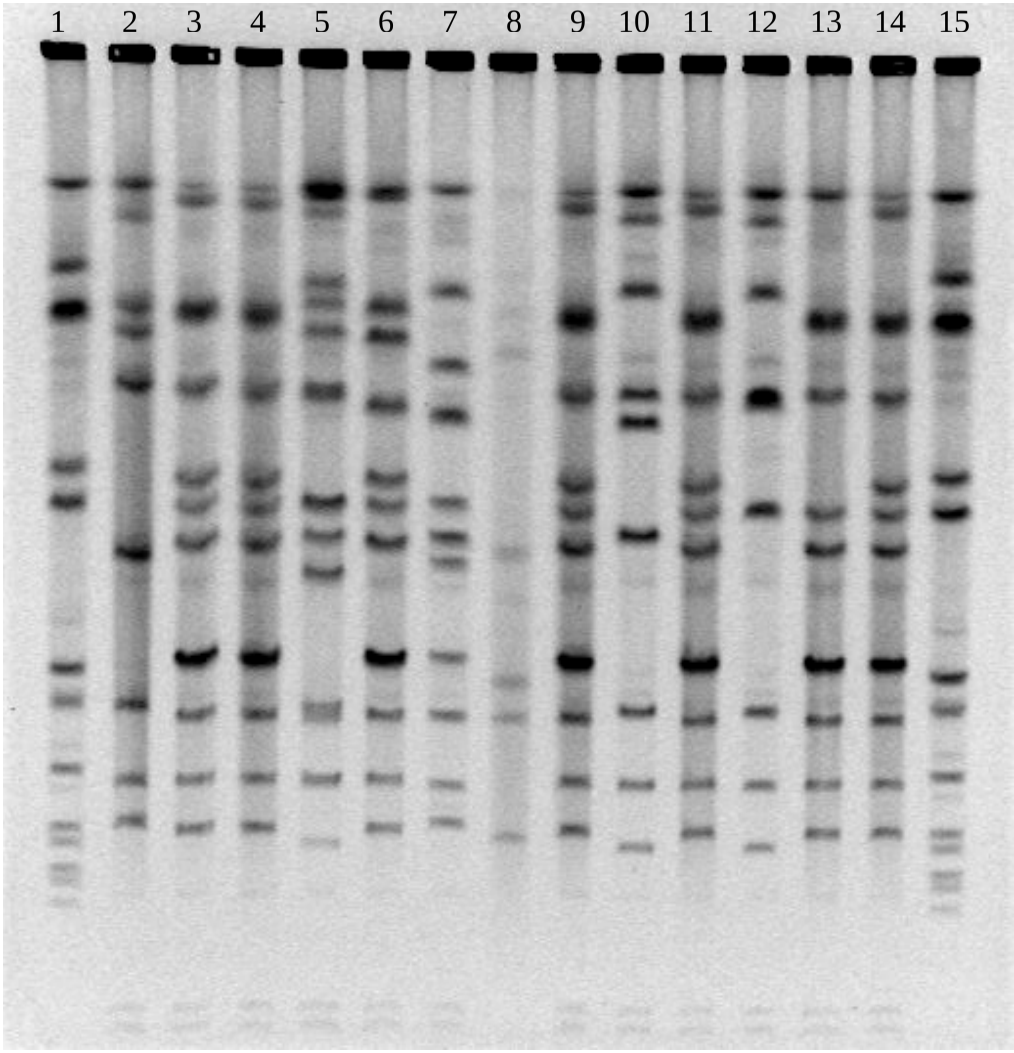
Lane	Source	Date	Group
1	H2446		
2	FAPC #821 Fecal	4/5/00	A
3	FAPC #814 Fecal	4/5/00	A
4	FAPC #2 Carcass	4/5/00	A
5	FAPC Holding Pen Drain	4/5/00	P
6	FAPC Hock Cutter	4/5/00	F
7	FAPC #6 Carcass	4/25/00	W
8	FAPC #7 Carcass	4/25/00	W
9	FAPC #8 Carcass	4/25/00	W
10	FAPC #2 Carcass	4/26/00	W
11	FAPC #3 Carcass	4/27/00	W
12	FAPC Holding Pen Drain	4/27/00	R
13	FAPC #1 Fecal	7/5/00	F
14	FAPC #1 Hide	7/5/00	F
15	H2446		

Figure 4: Pulsed Field Electrophoresis of *Listeria* spp. digested with *ASC* I; Pulsotypes A, P, F, W, R



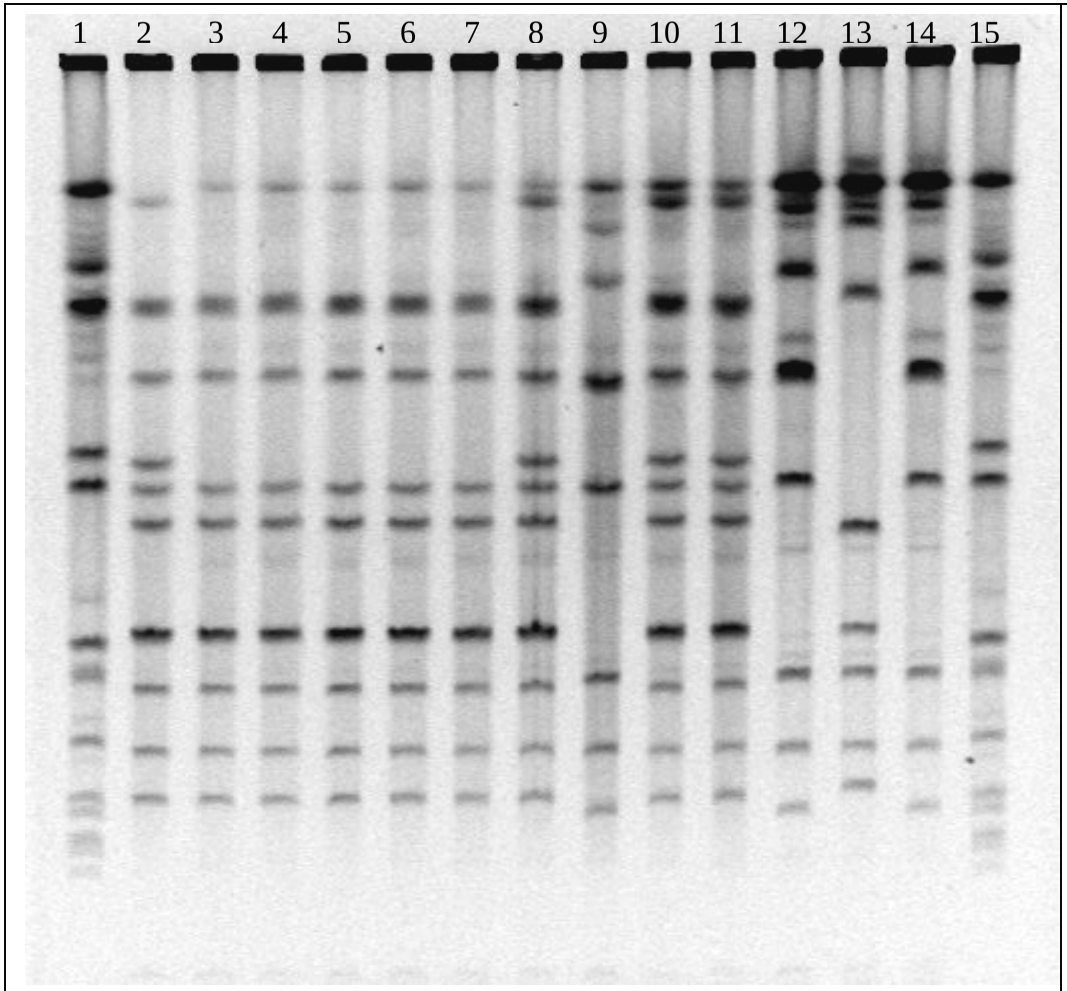
Lane	Source	Date	Group
1	H2446		
2	FAPC #2 Hide	7/5/00	H
3	FAPC #2 Fecal	7/5/00	F
4	FAPC #2 Carcass	7/5/00	B
5	FAPC #3 Hide	7/5/00	N
6	FAPC #4 Hide	7/5/00	N
7	FAPC #5 Hide	7/5/00	E
8	FAPC #6 Fecal	7/5/00	F
9	FAPC #6 Hide	7/5/00	N
10	FAPC #7 Fecal	7/6/00	F
11	FAPC #7 Hide	7/6/00	F
12	FAPC #8 Hide	7/6/00	N
13	FAPC #9 Hide	7/6/00	F
14	FAPC #10 Hide	7/6/00	F
15	H2446		

Figure 5: Pulsed Field Electrophoresis of *Listeria* spp. digested with *ASC* I; Pulsotypes H, F, B, N, E



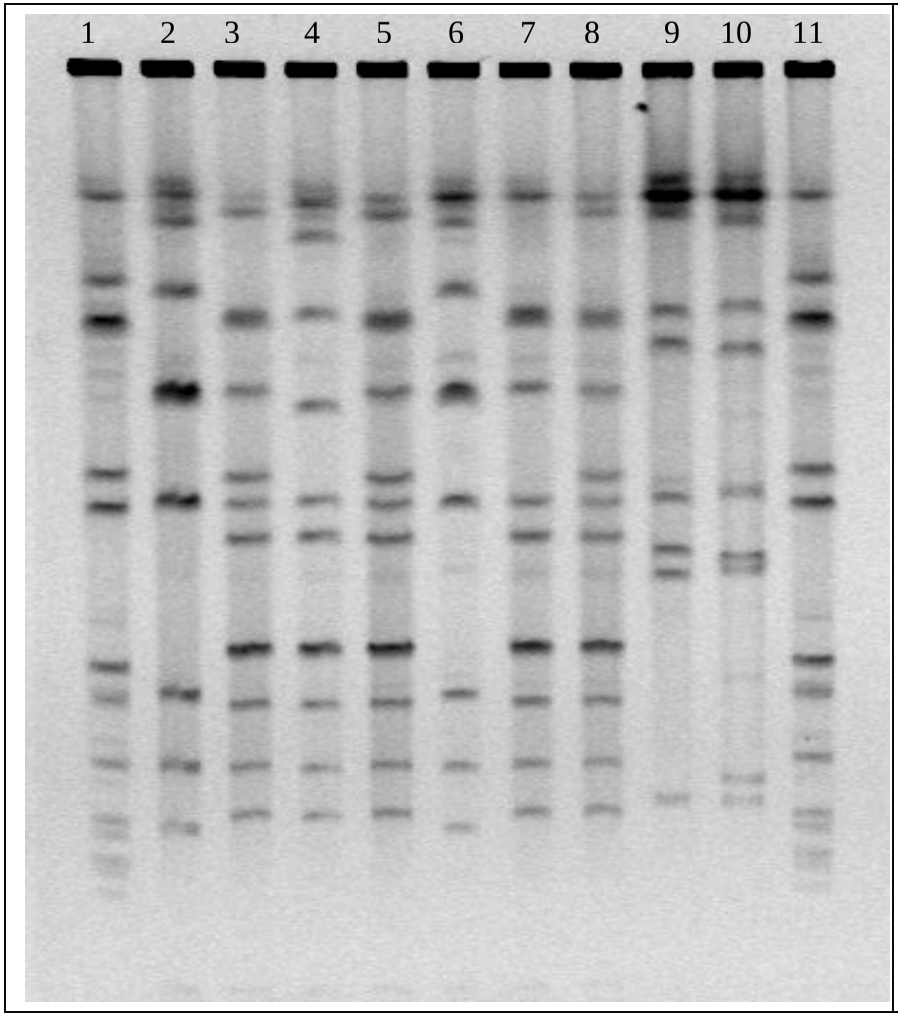
Lane	Source	Date	Group
1	H2446		
2	FAPC #11 Hide	7/6/00	A
3	FAPC #12 Hide	7/6/00	F
4	FAPC Bleeding Drain	7/5/00	F
5	FAPC Hot Cutter Drain	7/5/00	Q
6	FAPC Carcass Saw Drain	7/5/00	F
7	FAPC Evisceration Drain	7/5/00	I
8	FAPC Evisceration Drain	7/6/00	U
9	W.S. M.P. 5 Fecal	7/6/00	F
10	W.S. M.P. 10Fecal	7/6/00	P
11	FAPC #3 Fecal	8/9/00	F
12	FAPC #5 Fecal	8/9/00	N
13	FAPC #6 Fecal	8/9/00	E
14	FAPC #7 Fecal	8/10/00	F
15	H2446		

Figure 6: Pulsed Field Electrophoresis of *Listeria* spp. digested with *ASC* I; Pulsotypes A, F, Q, I, U, P, N, E



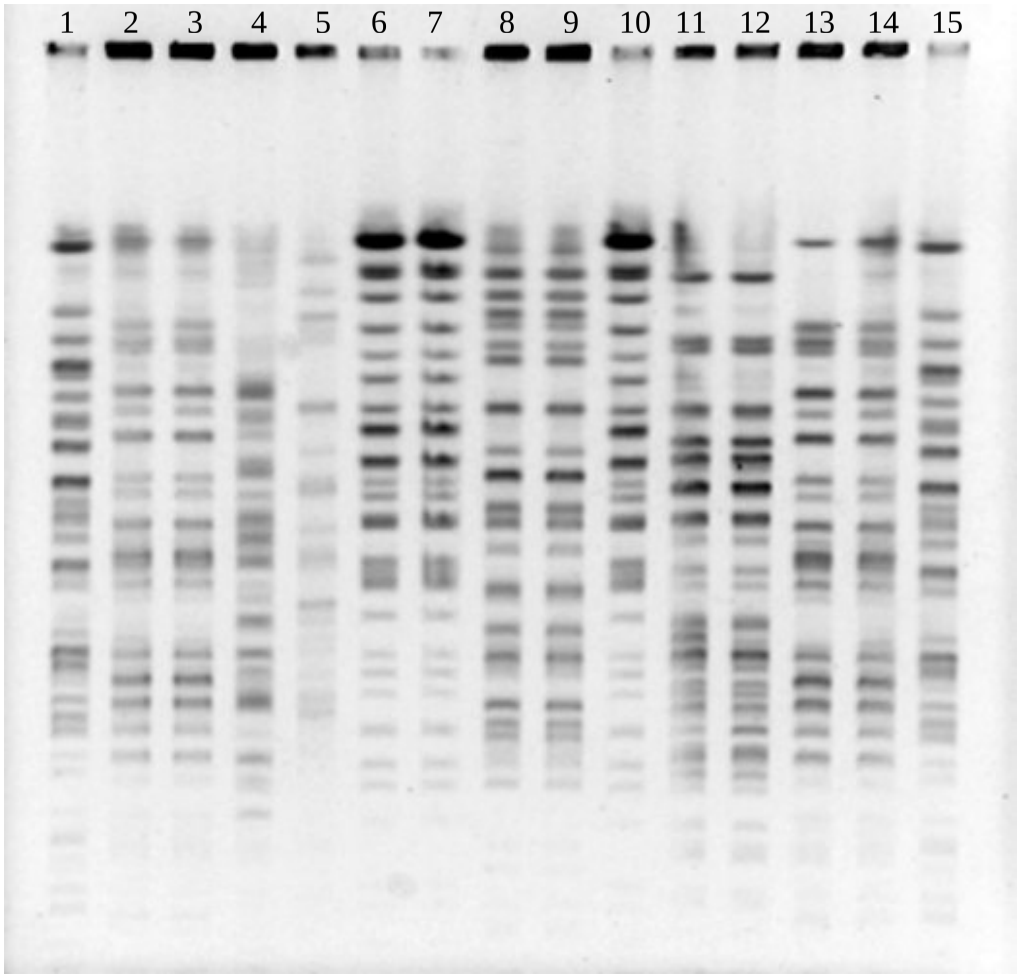
Lane	Source	Date	Group
1	H2446		
2	FAPC #8 Fecal	8/10/00	F
3	FAPC #9 Fecal	8/10/00	E
4	FAPC #10 Fecal	8/10/00	E
5	FAPC #11 Fecal	8/10/00	E
6	FAPC #12 Fecal	8/10/00	E
7	FAPC #12 Carcass	8/10/00	E
8	FAPC #1 Hide	8/9/00	F
9	FAPC #2 Hide	8/9/00	N
10	FAPC #3 Hide	8/9/00	F
11	FAPC #4 Hide	8/9/00	F
12	FAPC #5 Hide	8/9/00	N
13	FAPC #6 Hide	8/9/00	L
14	FAPC #7 Hide	8/10/00	N
15	H2446		

Figure 7: Pulsed Field Electrophoresis of *Listeria* spp. digested with *ASC* I; Pulsotypes F, E, N, L



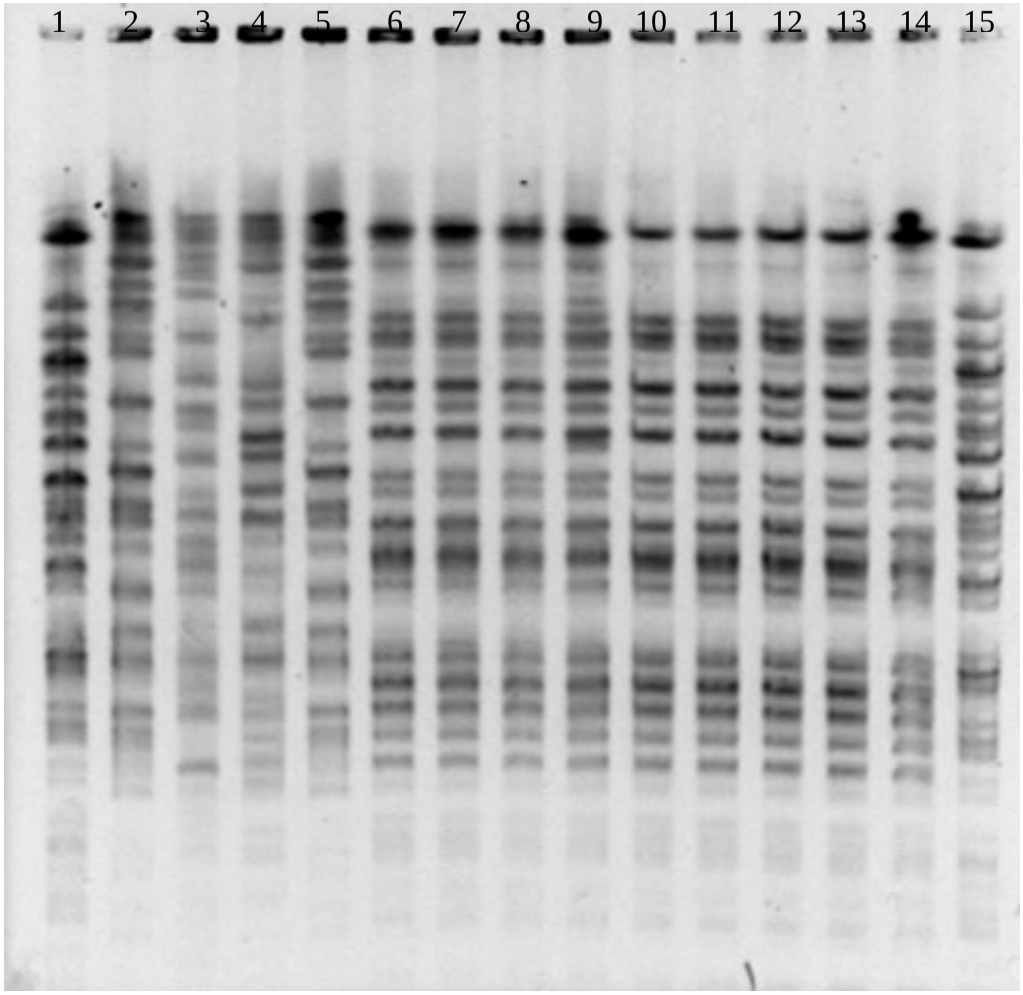
Lane	Source	Date	Group
1	H2446		
2	FAPC #8 Hide	8/10/00	N
3	FAPC #9 Hide	8/10/00	N
4	FAPC #10 Hide	8/10/00	G
5	FAPC #11 Hide	8/10/00	F
6	FAPC #12 Hide	8/10/00	N
7	FAPC #13 Hide	8/10/00	E
8	FAPC #5 Hide	8/17/00	F
9	FAPC Eviseration Drain	8/15/00	S
10	FAPC Hock Cutter Drain	8/15/00	S
11	H2446		

Figure 8: Pulsed Field Electrophoresis of *Listeria* spp. digested with *ASC* I; Pulsotypes N, G, F, E, S



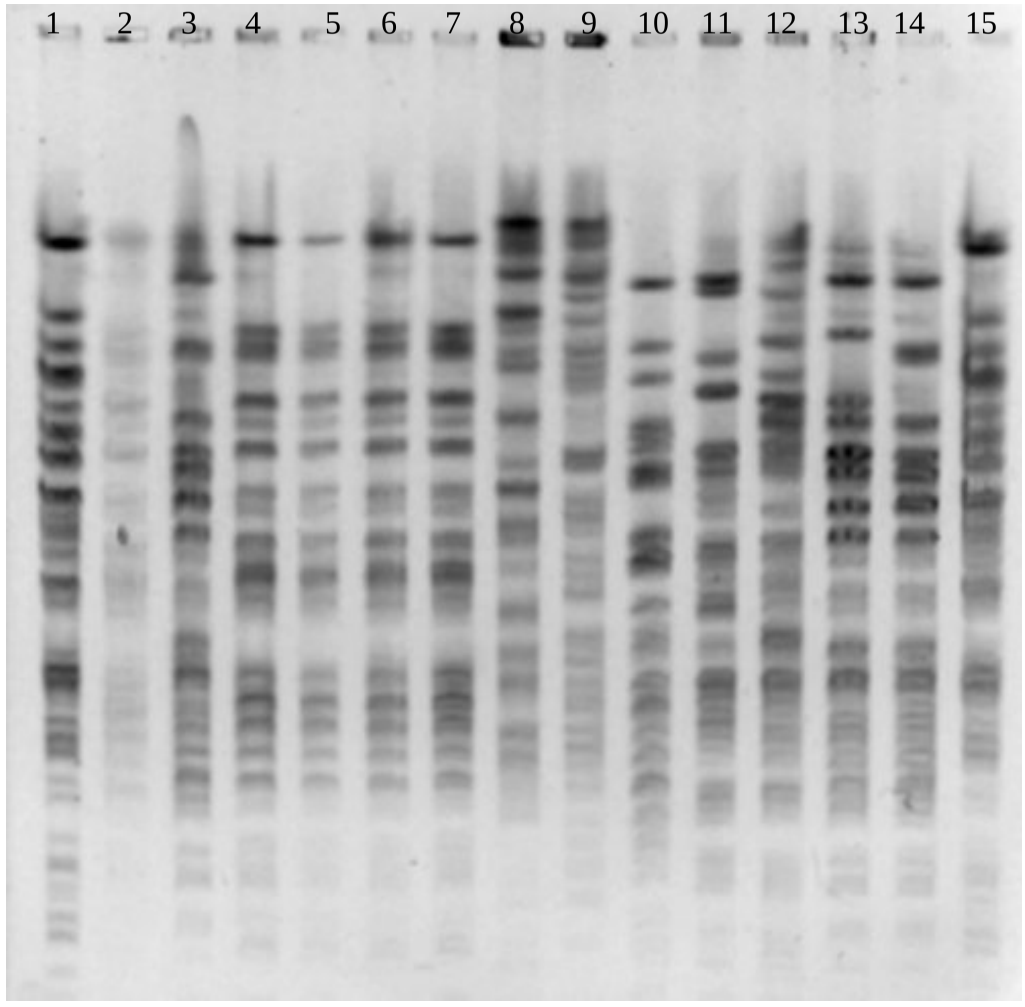
Lane	Source	Date	Group
1	H2446		
2	W.S. N 1 Fecal	4/12/99	S
3	W.S. N 3 Fecal	4/12/99	S
4	W.S. N 16 Fecal	5/26/99	V
5	W.S. N 19 Fecal	5/26/99	Y
6	Non-*Listeria* Spp.		
7	Non-*Listeria* Spp.		
8	W.S. N 23 Fecal	6/9/99	Z
9	W.S. N 25 Fecal	6/9/99	Z
10	Non-*Listeria* Spp.		
11	W.S. N 30 Fecal	6/9/99	E
12	W.S. N 32 Fecal	6/9/99	I
13	W.S. N 1 Fecal	6/15/99	S
14	W.S. N 21 Fecal	6/15/99	Z
15	H2446		

Figure 9: Pulsed Field Electrophoresis of *Listeria* spp. digested with *Sma* I; Pulsotypes S, V, Y, Z, E, I



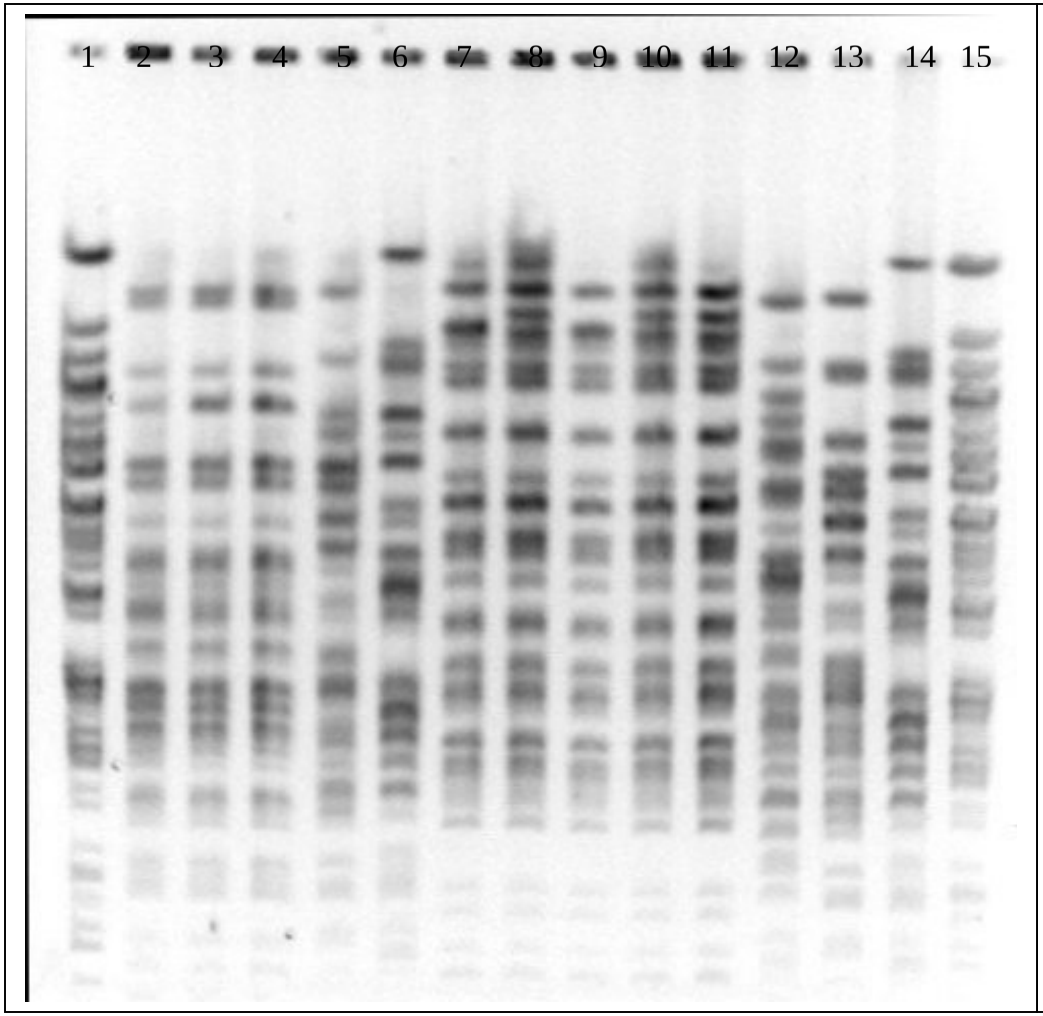
Lane	Source	Date	Group
1	H2446		
2	W.S. N 19 Fecal	6/15/99	S
3	W.S. S 5/6 Water	8/11/99	Q
4	FAPC Red Angus Fecal	10/7/99	K
6	FAPC White Face Carcass	10/14/99	Z
6	W.S. Direct Fecal	11/8/99	S
7	W.S. Direct Fecal	11/8/99	S
8	W.S. Direct Fecal	11/8/99	S
9	W.S. Direct Fecal	11/8/99	S
10	W.S. Direct Fecal	11/8/99	S
11	W.S. Direct Fecal	11/8/99	S
12	W.S. Direct Fecal	11/8/99	S
13	W.S. Direct Fecal	11/8/99	S
14	W.S. Direct Fecal	11/8/99	S
15	H2446		

Figure 10: Pulsed Field Electrophoresis of *Listeria* spp. digested with *Sma* I; Pulsotypes S, Q, K, Z



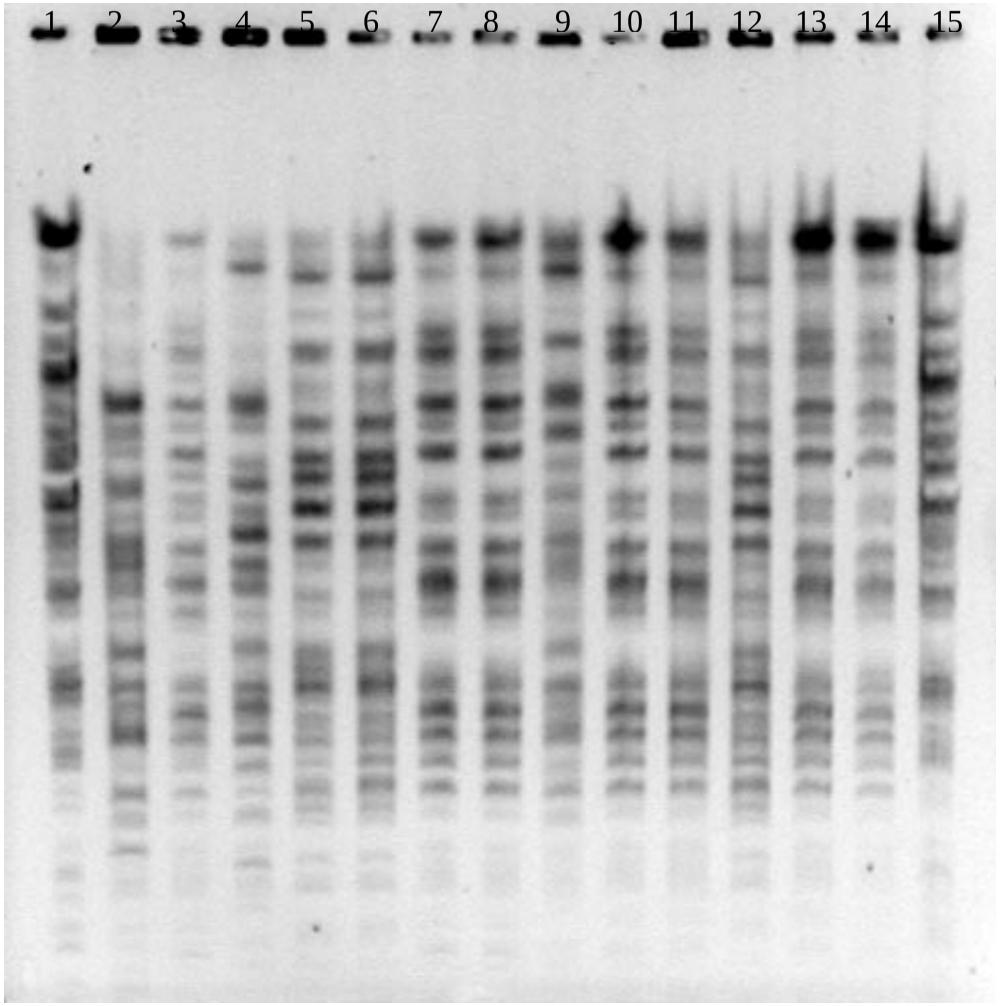
Lane	Source	Date	Group
1	H2446		
2	W.S. Direct Fecal	11/8/99	R
3	W.S. Direct Fecal	11/8/99	G
4	FAPC #2 Fecal	2/11/00	S
5	FAPC #3 Fecal	2/11/00	S
6	FAPC #4 Fecal	2/11/00	S
7	FAPC #3 Fecal	2/15/00	S
8	W.S. N MP 13/14 Fecal	4/16/00	Z
9	W.S. N MP 18 Fecal	4/16/00	AA
10	FAPC #815 Fecal	3/28/00	B
11	FAPC Hock Cutter Drain	3/28/00	C
12	FAPC Evisceration Drain	3/28/00	X
13	FAPC Holding Pen Drain	3/28/00	J
14	FAPC #3Fecal	4/5/00	P
15	H2446		

Figure 11: Pulsed Field Electrophoresis of *Listeria* spp. digested with *Sma* I; Pulsotypes R, G, S, Z, AA, B, C, X, J, P



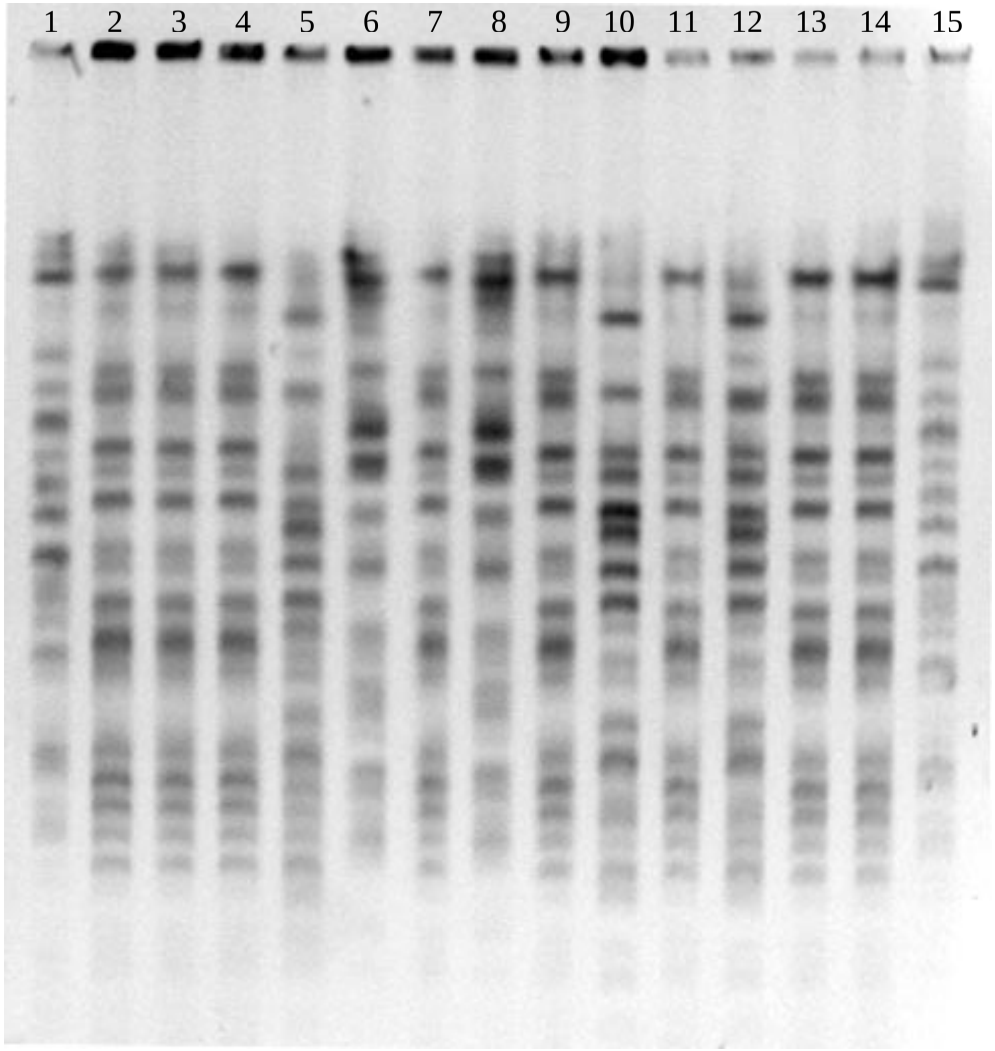
Lane	Source	Date	Group
1	H2446		
2	FAPC #821 Fecal	4/5/00	D
3	FAPC #814 Fecal	4/5/00	D
4	FAPC #2 Carcass	4/5/00	D
5	FAPC Holding Pen Drain	4/5/00	M
6	FAPC Hock Cutter	4/5/00	S
7	FAPC #6 Carcass	4/25/00	Z
8	FAPC #7 Carcass	4/25/00	Z
9	FAPC #8 Carcass	4/25/00	Z
10	FAPC #2 Carcass	4/26/00	Z
11	FAPC #3 Carcass	4/27/00	Z
12	FAPC Holding Pen Drain	4/27/00	A
13	FAPC #1 Fecal	7/5/00	H
14	FAPC #1 Hide	7/5/00	S
15	H2446		

Figure 12: Pulsed Field Electrophoresis of *Listeria* spp. digested with *Sma* I; Pulsotypes D, M, S, Z, A, H



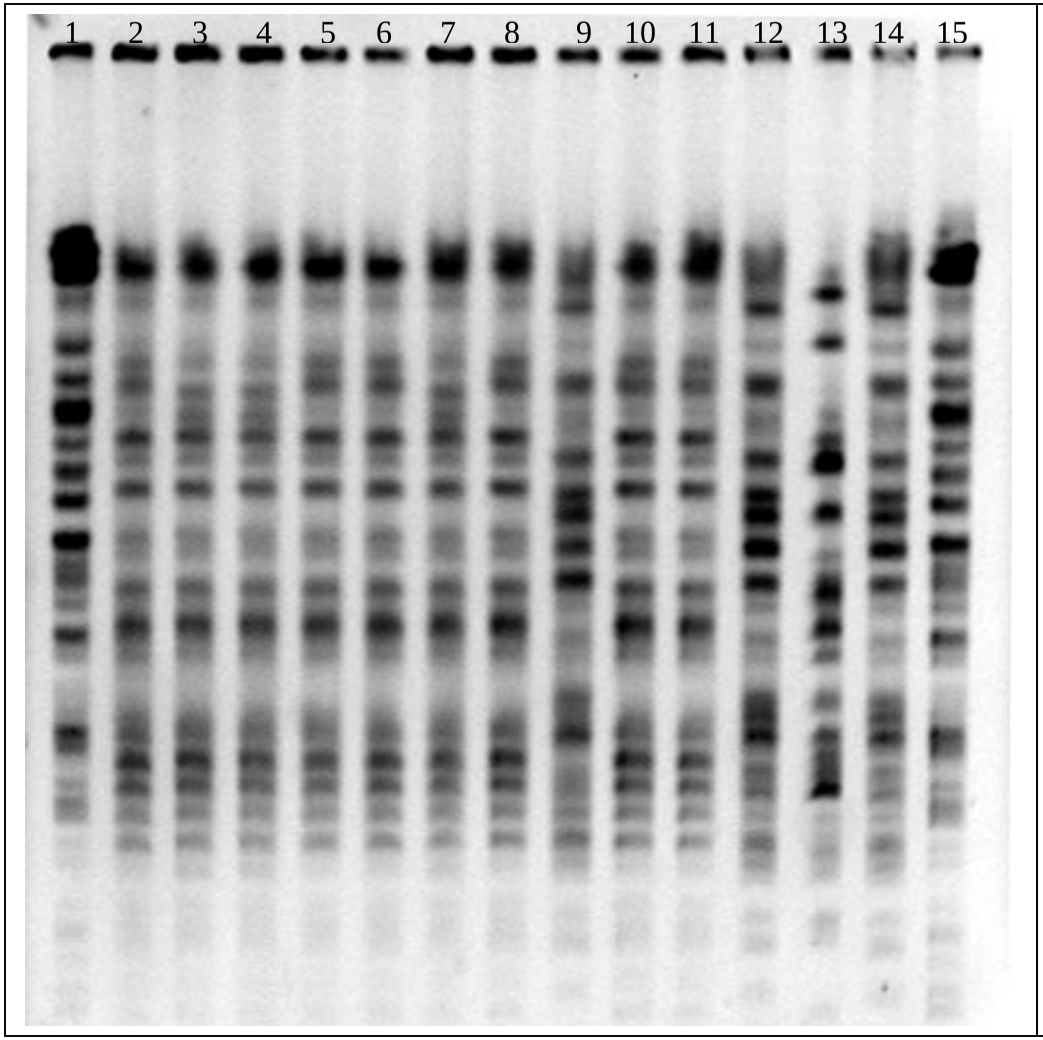
Lane	Source	Date	Group
1	H2446		
2	FAPC #2 Hide	7/5/00	U
3	FAPC #2 Fecal	7/5/00	T
4	FAPC #2 Carcass	7/5/00	O
5	FAPC #3 Hide	7/5/00	F
6	FAPC #4 Hide	7/5/00	G
7	FAPC #5 Hide	7/5/00	S
8	FAPC #6 Fecal	7/5/00	S
9	FAPC #6 Hide	7/5/00	H
10	FAPC #7 Fecal	7/6/00	S
11	FAPC #7 Hide	7/6/00	S
12	FAPC #8 Hide	7/6/00	F
13	FAPC #9 Hide	7/6/00	S
14	FAPC #10 Hide	7/6/00	S
15	H2446		

Figure 13: Pulsed Field Electrophoresis of *Listeria* spp. digested with *Sma* I; Pulsotypes U, T, O, F, G, S, H



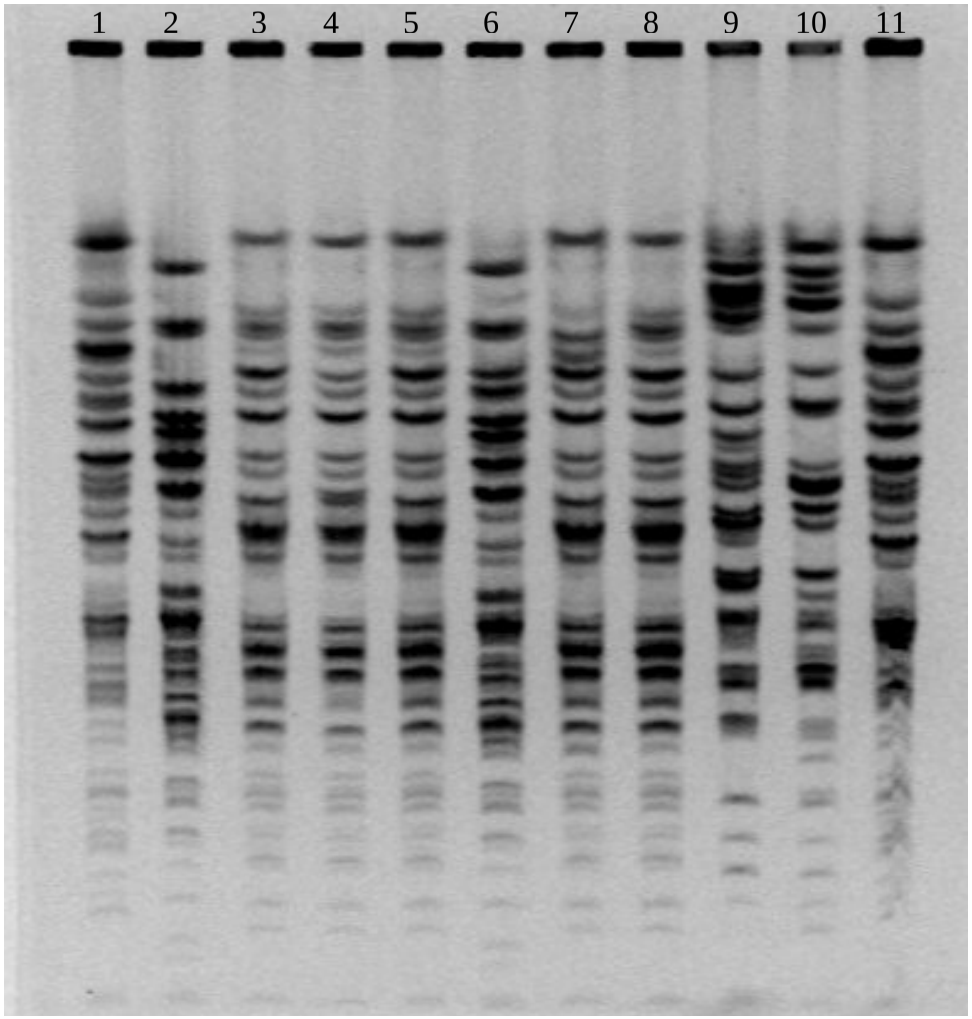
Lane	Source	Date	Group
1	H2446		
2	FAPC #11 Hide	7/6/00	S
3	FAPC #12 Hide	7/6/00	S
4	FAPC Bleeding Drain	7/5/00	S
5	FAPC Hock Cutter Drain	7/5/00	E
6	FAPC Carcass Saw Drain	7/5/00	BB
7	FAPC Evisceration Drain	7/5/00	S
8	FAPC Evisceration Drain	7/6/00	BB
9	W.S. M.P. 5 Fecal	7/6/00	S
10	W.S. M.P. 10Fecal	7/6/00	L
11	FAPC #3 Fecal	8/9/00	S
12	FAPC #5 Fecal	8/9/00	G
13	FAPC #6 Fecal	8/9/00	S
14	FAPC #7 Fecal	8/10/00	S
15	H2446		

Figure 14: Pulsed Field Electrophoresis of *Listeria* spp. digested with *Sma* I; Pulsotypes S, E, BB, L, G



Lane	Source	Date	Group
1	H2446		
2	FAPC #8 Fecal	8/10/00	S
3	FAPC #9 Fecal	8/10/00	S
4	FAPC #10 Fecal	8/10/00	S
5	FAPC #11 Fecal	8/10/00	S
6	FAPC #12 Fecal	8/10/00	S
7	FAPC #12 Carcass	8/10/00	S
8	FAPC #1 Hide	8/9/00	S
9	FAPC #2 Hide	8/9/00	G
10	FAPC #3 Hide	8/9/00	S
11	FAPC #4 Hide	8/9/00	S
12	FAPC #5 Hide	8/9/00	F
13	FAPC #6 Hide	8/9/00	W
14	FAPC #7 Hide	8/10/00	F
15	H2446		

Figure 15: Pulsed Field Electrophoresis of *Listeria* spp. digested with *Sma* I; Pulsotypes S, G, F, W



Lane	Source	Date	Group
1	H2446		
2	FAPC #8 Hide	8/10/00	G
3	FAPC #9 Hide	8/10/00	S
4	FAPC #10 Hide	8/10/00	S
5	FAPC #11 Hide	8/10/00	S
6	FAPC #12 Hide	8/10/00	G
7	FAPC #13 Hide	8/10/00	S
8	FAPC #5 Hide	8/17/00	S
9.	FAPC Evisceration Drain	8/15/00	CC
10.	FAPC Hock Cutter Drain	8/15/00	CC
11	H2446		

Figure 16: Pulsed Field Electrophoresis of *Listeria* spp. digested with *Sma* I; Pulsotypes G, S, CC

VITA

William Dean Quimby

Candidate for the Degree of

Master of Science

Thesis: INCIDENCE OF LISTERIA SPECIES IN CATTLE, HOLDING FACILITIES, FECES, HIDES AND FINISHED BEEF CARCASSES

Major Field: Food Science

Biographical:

Personal Data: Born in Enid Oklahoma, on December 20, 1974, the son of Gene and Carol Quimby.

Education: Graduated from Garber High School, Garber, Oklahoma in May 1993; received Associate of Arts in Animal Science from Northeastern Oklahoma A&M in May 1995 and received Bachelor of Science in Pre-Veterinary Medicine in December 1997. Completed the requirements for the Master of Science degree with a major in Food Science at Oklahoma State University in December 2004.

Experience: Raised on a farm near Garber Oklahoma. Owned and operated Quimby Tire Service from 1991 to 1995; employeed by Oklahoma State University Food and Ag Products Center as an undergraduate, a graduate research assistant and a research specialist from 1997 to 2001; employed by Bar-S Foods, Company as HACCP Coordinator and Quality Assurance Manager from 2001 to present.

Professional Memberships: American Meat Institute, American Management Association