Food Safety

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Food Safety and HACCP in the Foodservice Industry

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Education is the key to establishing a food safety program in the foodservice industry. In addition, the Hazard Analysis Critical Control Point system can play an important role in managing a foodservice operation. Virginia Cooperative Extension Service, in conjunction with the Virginia Department of Health, has instituted a certification program for industry managers in foodservice sanitation. Survey results from managers taking the course indicate that:

- 97% have trained or shared knowledge gained with their employees;
- 92% have incorporated new food handling practices;
- 58% have received a higher health inspection score;
- 91% believe that other managers would benefit from this training; and
- 95% believe that this training should be mandatory in Virginia.

Consumer Attitudes Toward Food Safety

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A mail survey was administered to 1,100 households in Alabama to determine consumer attitudes about food safety. A total of 462 surveys were returned with 53% of the respondents less than 45 years of age, 91% white, 54% male, 64% with an annual income of more than $25,000 and 67% with at least some college education. Average family size of respondents was three members. Most consumers (83%) expressed at least a moderate level of concern about the safety of the food supply, feeling that most foods were mishandled in the food processing industry ($P<.05$). Over 66% indicated that foods in general are well-inspected but that inspection needs improving, while 29% felt that beef is adequately inspected. However, 92% of all consumers rated beef as somewhat or very safe ($P<.05$). Specific concerns related to bacteria, nutrition and chemical residues. Most consumers reported receiving their food safety information from the media ($P<.05$). Over 45% reported consuming beef at least six times in a two-week period, with consumption decreasing as age increased ($P<.05$). Almost half of the consumers surveyed desired a medium-well degree of doneness for beef products they consumed ($P<.05$). Beef ranked second only to vegetables as consumers' choice of nutritional foods.

Effects of Environmental Stress on Antimicrobial Drug Resistance of *Escherichia coli* of the Intestinal Flora of Swine

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Antimicrobial resistance of fecal *E. coli* obtained from finisher pigs exposed to controlled temperatures of 34°C (heat stress) were examined. Twelve animals were assigned to four experimental groups: (a) control, (b) exposed for 8 hours and slaughtered immediately afterward, (c) exposed for 24 hours and slaughtered immediately afterward and (d) exposed 24 hours but slaughtered after 1 week. Samples were collected from different segments of the intestinal tract (jejunum, ileum, ileocecal valve, cecum, spiral colon, and rectum) as well as carcasses at the packing plant. Samples were plated in Tergitol-7 agar and 10 *E. coli* suspected colonies were randomly selected from each segment and identified by biochemical reactions. Antibiograms were assayed by an agar dilution technique. The predominant antimicrobial resistance patterns were observed to change within 8 hours following heat stress. In the lower intestinal tract, the percentage of isolates resistant to Ampicillin changed from 20% to 54% in the spiral colon and from 10% to 43% in the rectum. The percentage of isolates resistant to Tetracycline changed from 49% to 79% in the spiral colon and from 42% to 65% in the rectum. *E. coli* isolated from carcasses of stressed swine were twice as likely to be resistant to Tetracycline than were bacteria from carcasses of non-stressed swine (80% vs. 40%). These results demonstrate that environmental (heat) stress increases the number of Ampicillin- and Tetracycline-resistant *E. coli* in the lower intestinal tract and consequently increases shedding of these organisms into the environment and food chain.

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Listeria in Meats: Current Issues in Europe

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According to a list assembled in France for the WHO (1991), about 300 laboratories are working worldwide on Listeria. Of these, about 225 laboratories are working on foods and *Listeria monocytogenes* (Lm), since foods are known to be the major source of listeriosis in humans.

Many foods—including meat and meat products—contain Lm frequently, but generally in low numbers. Probably many consumers ingest small numbers of Lm every day with their food, without adverse effects. However, high numbers of Lm could pose a risk, especially for pregnant women and immunocompromised persons.

Current issues with Lm in food in Europe are: (1) identification of foods at risk (e.g. pâté); (2) tolerances for Lm in foods, taking the minimal infectious dose into account; (3) inactivation of Lm in slow-heated meats (with emphasis on heat-shock proteins); (4) avoidance of recontamination of heated meats (by clean-rooms and regulations for machinery); and (5) inhibition of Lm growth in meat products (by protective cultures, bacteriocins or acetate addition).

Applications of Bacteriocins from Lactic Acid Bacteria in Combatting Pathogens and Spoilage Bacteria in Meat

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Bacteriocins from lactic acid bacteria are bacteriocidal proteins of small molecular weight, which vary greatly in host range. While most bacteriocins have a narrow host range, some such as nisin from *Lactococcus lactis* and pediocin from *Pediococcus acidilactici* have relatively wide host range, that includes many bacteria associated with food spoilage and foodborne diseases. Because of this, there has been an increase in interest of their possible use as food biopreservatives. Because these bacteriocins are produced by food-grade bacteria which have been consumed by humans for thousands of years without any known adverse effect, incorporation of their cells or metabolites in food may be accepted by the industries, regulatory agencies and consumers. However, before a bacteriocin can be used in a food, its antibacterial effectiveness, safety, economical production, stability and other important characteristics need to be investigated and discussed.

Pediocin AcH, from *Pediococcus acidilactici* H, is bacteriocidal against many Gram-positive pathogenic and spoilage bacteria important in foods. It is a small protein, non-toxic, heat stable and is destroyed by the proteolytic enzymes of the gastrointestinal tract. Most of the characteristics of this protein that are necessary for its use in food are known. It is fairly stable, can be produced economically, and can be incorporated in foods without any adverse effect. Pediocin AcH, like other bacteriocins of lactic acid bacteria, is structurally, functionally and chemically different from therapeutic antibiotics and thus should be accepted as an antibacterial protein, produced by safe food-grade bacteria, for use as a food biopreservative.

Non-Proteolytic Clostridium Botulinum Growth in Sous Vide Processed Muscle Foods

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Clostridium botulinum is an important bacterial pathogen to humans because of the extreme potency of the neurotoxin that this organism produces, and because it is a spore-forming organism which can survive normal cooking processes. Non-proteolytic strains of *C. botulinum* are capable of growth at refrigeration temperatures and once the spores survive the heat process, they are capable of making a product toxic in a relatively short period of time. In this study, an evaluation was made of the *sous vide* (under vacuum) process, which cooks food at a relatively low temperature for a long period to minimize thermal changes in the food. The foods inoculated with *C. botulinum* were pork chops, boneless chicken breasts, beef steaks and salmon steaks.

Spores of non-proteolytic *Clostridium botulinum* were able to survive through all cooking processes tested, and to germinate and produce toxin at temperatures of either 8 or 12°C in all foods and at 4°C in three of the four foods. Two of the four foods had produced toxic samples at 4°C by 21 days. Foods became toxic much quicker when incubated at either 8 or 12°C, with all four foods having toxic samples by 8 days of storage. At lease one toxic sample of each of the four foods was rated as consumable by at least one panelist evaluating by odor whether the food was fit for human consumption. Spoilage was not an indicator of safety.

Evaluation of Pathogenic and Spoilage Organisms on Pork Carcasses During Slaughter and Fabrication

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The contamination of pork carcasses by pathogenic and spoilage bacteria at four sites along the slaughter and fabrication process was studied. Three typical pork slaughtering facilities were sampled after singeing and polishing of carcasses, after the final shower on the slaughter floor, after an 18-24 hour chill, and after deboning and trimming but just prior to vacuum packaging of boneless loins. Microbial counts were obtained using a moistened swab technique and a 100 cm^2 template. Carcasses were sampled at the midpoint of the loin during normal working conditions. The pork carcasses and boneless loins were sampled for the pathogens: *Salmonella* spp., *Staphylococcus aureus, Listeria monocytogenes, Yersinia enterocolitica and Clostridium*

perfringens, as well as the spoilage type psychrotrophs, coliforms, anaerobic mesophiles, aerobic mesophiles and lactic acid bacteria.

Salmonella, S. aureus and *L. monocytogenes* were the most prevalent pathogens found. A significant linear increase was found for *S. aureus* as carcasses progressed from slaughter to the fabrication processes ($P<0.05$). Trimming of pork loins in fabrication reduced the number of *S. aureus* isolates. *L. monocytogenes* was found after singeing, after the final wash on the slaughter floor and after 24 hours chill. *Y. enterocolitica* was found after the final wash on the slaughter floor. *C. perfringens* was found after 24 hours chill and on the fabrication floor. These results show that post-slaughter contamination of pork carcasses with pathogenic organisms poses a threat to the safety of fresh product since some pathogens are still present after 36 days of refrigeration.

For the psychrotrophs, coliforms, and lactic acid bacteria, the loins were found to be significantly ($P<0.05$) more contaminated during the fabrication and cutting processes (Site 4). The area after singeing and polishing (Site 1) established the initial microbial load on the carcass. The carcasses sampled at the second site (after the final rinse on the slaughter floor) and the third site (after an 18-24 hour chill) were found to have significantly ($P<0.05$) reduced counts when compared to the first and fourth sites. For the mesophilic anaerobes and aerobes, the greatest amount of contamination was found at Site 1 (after singeing and polishing, $P<0.01$).

Characteristics of Bacteriocins from Lactic Acid Bacteria for Use in Meat and Meat Products

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Refrigeration storage in combination with vacuum packaging has been a popular means of extending shelf-life of unprocessed (fresh) and processed (low heat) meat products. However, good manufacturing practices, effective vacuum packaging, use of low-oxygen permeable packaging materials and low storage temperature (close to 1°C) have provided selective growth advantages of several pathogenic and spoilage bacteria capable of growing at low temperature. Because these products are expected to have long shelf-life, even from a very low initial population they can reach high population to adversely affect the safety and shelf-life of these products. Temperature abuse (10° to 12°C), even for a few hours, can accelerate their growth, resulting in rapid loss of product quality. To overcome these problems, the regulatory agencies have advised to incorporate suitable preservative(s) as a secondary barrier.

Many preservatives that are used conventionally may not be acceptable by health-conscious consumers. Bacteriocins of food-grade safe lactic acid bacteria, that are small antibacterial proteins may be least objectionable for use in foods as biopreservatives to control the pathogenic and spoilage bacteria in refrigerated vacuum-packaged meat products. However, only a few bacteriocins (such as nisin and pediocin AcH) have been tested to have relatively wide antibacterial spectrum. Even then they are not normally effective against Gram-negative bacteria and all Gram-positive bacterial strains; even in sensitive Gram-positive strains, there are variants that are resistant to bacteriocins. As bacteriocins are not bacteriostatic, the resistant cells can multiply to reach a high population during long storage at refrigeration temperature and affect the quality of a product.

Pediocin AcH, with relatively wide antibacterial spectrum, can be used as a biopreservative in vacuum-packaged refrigerated meat products to reduce spoilage and health hazard. However, it is not normally potent against Gram-negative and some Gram-positive spoilage and pathogenic bacteria which can be important in these products. This deficiency can be reduced by making pediocin-based biopreservatives that have wide host range not only against Gram-positive but also Gram-negative bacteria that can be present and reduce acceptance quality of their products. The results presented here indicate effectiveness of such biopreservatives.

Bacterial Contaminants of Retail Fresh Meat and Meat Products

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Fresh meat and meat products were purchased at local supermarkets and cultured for bacterial contaminants. A total of 612 meats were purchased from the following categories: fresh chilled meats; ground meats; variety meats; fresh sausages; cured, partially cooked meat products; cured cooked meat products; cured fermented meat products; canned sterilized meat products; sandwich spreads and meat salads; and cooked foods containing meats.

The principal sources of bacteria in meats and meat products are three-fold: animals and poultry, animal handlers and the environment. In healthy animals, the muscle tissues and internal organs classed as edible are sterile. Skin, hair and feathers of animals and poultry, the hands or gloves of handlers; knives, saws, slicers and tables; and the air are not sterile. These sources deposit bacteria on products which were sterile in the live animal. Standard Plate Cultures for aerobic bacteria were performed to measure overall bacterial contamination from all sources: animals, human handlers and the environment. Coliform counts for *Escherichia coli* and *Staphylococcus aureus* counts were assayed as indicators of contamination, originating from slaughter animals and human contamination respectively.

If meats and meat products were warm and moist enough, contaminating bacteria grew. Spoilage bacteria usually grew faster than pathogenic bacteria. Inhibition of spoilage bacteria may permit pathogenic bacteria to grow, which is a factor often unsuspected by consumers.

Since 25% of food borne diseases are traced to meats and meat products, the major critical control points are in slaughter sanitation, care in handling and temperature control.

Modeling the Growth of *Salmonella* During Carcass Cooling

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Lag and generation times for the growth of *Salmonella typhimurium* on sterile lean beef were modelled as functions of cooling time under various carcass chilling scenarios. Gompertz growth models were fit to the logic colony counts over time at each of six temperatures in the range of 15°C to 40°C. Lag and generation times were defined to be the points at which the second and first derivatives, respectively of each growth curve attained a maximum. Generation time and lag time parameters were modeled as functions of temperature using exponential decay models. The models were applied to typical beef carcass cooling scenarios to predict the potential growth of *S. typhimurium* during the cooling of beef. Validation studies indicated no significant difference between the observed and predicted bacterial populations on inoculated lean and fat beef tissues cooled at either 6°C or 9°C per hour.

Temperature End-Point Indicators to Assure Product Safety

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Inadequate cooking is commonly cited as a contributing factor in foodborne disease outbreaks caused by meat and poultry products. To prevent potential foodborne diseases caused by underprocessing, the United States Department of Agriculture, Food Safety and Inspection Service (USDA-FSIS) requires that certain meat and poultry products be cooked to safe end-point temperatures (EPT) under Title 9 of the Code of Federal Regulations (CFR).

Currently, FSIS is using a protein "Coagulation Test" for monitoring the EPT of beef and pork products heat-processed to temperatures lower than 65°C and for uncured and cured poultry products; a residual "Acid Phosphatase Activity Method" for determining the EPT of imported canned hams, canned picnics and canned luncheon meat; and a "Bovine Catalase Test" for detection of underprocessing of rare roast beef and cooked beef. Additional methods that have been investigated by researchers around the world include: dominant spectral wavelengths, fluorescence, NIR, DSC, electrophoresis HPLC techniques, ELISA techniques, temperature sensitive indicator disks and residual enzyme activity procedures. However, many of these methods are inaccurate, time consuming, subjective, or require a large number of reagents and/or sophisticated scientific equipment and, thus, have not been adopted for widespread use.

Research being conducted at the Russell Research Center toward the development of new and/or improved methods for determining the EPT of meat and poultry products include: HPLC techniques for characterizing components generated in heat-treated muscle tissue, an objective "Catalase Test" for monitoring the EPT of rare roast beef and cooked beef, a specific component of meat juice color as an EPT marker for imported cooked beef, pyruvate kinase activity in imported canned hams and domestic-type beef and poultry products, residual acid phosphatase activity via dedicated instrumentation, characterization of heat-treated muscle proteins by HPLC diode array analysis, factors that may alter the coagulation temperature of filtrates used in the "Coagulation Test" and the development of an objective procedure for expressing the results of the coagulation test and an evaluation of various test kits as potential EPT indicators or markers.

Adhesion of Bacteria to Equipment and Product Surfaces

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Meat processing equipment and product surfaces provide attachment sites for pathogenic and spoilage bacteria and are a means of transfer of these organisms from contaminated sources to clean recipients. Transfer and adhesion of bacteria to surfaces are multifactorial, with interaction of physical and chemical factors involving the surfaces of the bacteria, processing equipment and meat products. Physical factors include entrapment in irregular surfaces, electrostatic attraction, hydrophobic and hydrophilic attachments, and surface/tension/hydrodynamic effects. Chemical factors include attachment through specific chemical bonds between the bacteria and other surfaces and the microbial growth with formation of biofilms. Reduction of these means of microbial contamination through HACCP involves an examination of incoming carcasses for level and type of microbial contamination, environmental conditions in the processing plant, the nature of all processing surfaces, and the procedures for plant sanitation during the various stages of conversion into final products and their subsequent storage.

Survival of *Salmonella* Species *Staphylococcus Aureus* and *Listeria Monocytogenes* Heated by Microwave Energy in Food Systems of Various Complexity

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Five food systems (UHT milk, beef broth, pudding, cream sauce and liquid whole egg) with various chemical and

physical properties were examined in an effort to determine factors important in achieving uniform temperatures within foods heated by microwave energy. These properties were examined for their influence on the thermotolerance of *Salmonella* species, *Listeria monocytogenes* strains Scott A and V7 and *Staphylococcus aureus* ATCC 25923 heated in a 700 W microwave oven. Proximate analysis was performed on all food systems to relate their chemical composition to temperatures achieved during heating, monitored by fluoroptic thermometry and to the destruction of bacteria. The final mixed mean temperature achieved by most systems was 60°C for *Salmonella* spp. and *L. monocytogenes* and 65°C for *S. aureus*. The amount of destruction of *Salmonella* spp. varied from 99.95% in UHT milk to 64.76% in beef broth. *L. monocytogenes* strains incurred the greatest amount of destruction in milk (99.56%), while the least amount of destruction was observed in cream sauce (95.17%). The greatest amount of destruction of *S. aureus* occurred in milk (99.25%), while the least amount of destruction occurred in cream sauce containing 1.0% NaCl (98.38%). The pH and a_w of the foods studied did not appreciably affect survival of *Salmonella* that were thermally stressed. Total ash content did not appear to be as important as sodium content in relation to the amount of bacterial destruction observed. Sodium greatly affected the uniformity of temperatures achieved within foods, and in turn, on the survival of bacteria present within the foods. As a trend, greater numbers of bacteria survived when heated in foods of higher sodium content. Increased recovery of bacteria in food containing greater concentrations of salt was determined not to be due to a lowering of water activity. The amount of thermal destruction of *Staphylococcus aureus* ATCC 25923 and *Listeria monocytogenes* strains Scott A and V7 in foods heated by microwave energy was estimated by calculations based upon their published thermal resistance values in food products and the food products time-temperature profile at 20 locations within the container. These predicted values were compared to experimentally determined bacterial destruction values, and underestimated experimentally determined bacterial destruction by approximately two $\log_{10}$ reductions.

Rapid Methods for Detecting Pathogens Using Modified Antibody Techniques

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The central challenge of the food microbiologist is to detect specific pathogens in a complex menstruum (food) among a background of a mixed population of other microorganisms. The pathogen of interest is often present in low numbers only a fraction as populous as the normal microflora of the food. Enrichment culturing offers a means of selecting for the outgrowth of specific classes of bacteria from mixed populations, thereby increasing the numbers of the pathogen relative to the background flora. Traditionally, an additional selective culture step is needed after primary enrichment to "fish out" and identify the organism of concern. It is at this step that using immunodiagnostics can greatly decrease analytical time and increase detection accuracy. Immunodiagnostic kits can answer the presence/absence question rapidly, thereby eliminating the need for further time-consuming culture steps. Immunodiagnostics are based on using a specific antibody that binds specifically to only one genus of bacteria. At least one kit is available that offers the potential for quantitative data (*Listeria* spp.) to be obtained in the same short time frame. As this poster will demonstrate, the gap between lengthy (several days) microbiological analysis and practical real-time analysis is closing. The purpose of this paper is to summarize features of some of the immunodiagnostics available to the food microbiologist specifically for *Listeria* spp. and *Salmonella* spp. At the time of presentation of this paper four manufacturers had commercially available tests that were readily available in the United States. These included BioControl Systems, Inc. (Assurance EIA-Salmonella, Assurance EIA-Listeria), Organon-Teknika Corp. (Listeria-Tek, Salmonella-Tek), Vicam (Listertest LIFT), Tecra Diagnostics (Salmonella Immunocapture, Salmonella Visual Immunoassay, Listeria Visual Immunoassay). Of these assays, the *Salmonella* spp. tests (except for the Immunocapture) had AOAC approval for food testing. The other tests all were in progress for AOAC approval. The only quantitive assay is the Listertest LIFT assay by Vicam. All of these assays require 2 to 2.5 days to complete and all require culture confirmation of positive results. The Listertest LIFT and the Salmonella Immunocapture do not require enrichment steps. The key advantages of immunodiagnostics include: short analysis time, reproductibility, ease of use, adaptability to US-FDA and USDA-FSIS methods, generally cost-effective when compared to culture methods, technical assistance availability, no radioisotopes needed, and reagent QA done by manufacturer. All of the immunodiagnostics listed offer the advantage of quicker negative results, allowing more expedient shipment of product vs. costly holding times spent waiting for lab results.

Effects of Post-Packaging Pasteurization on *Listeria Monocytogenes* in Precooked Vacuum Packaged Beef.

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Precooked beef loin chunks were inoculated separately with three strains of *Listeria monocytogenes* (Scott A, 101M, and 103M). Uninoculated chunks served as controls. All chunks were vacuum packaged after inoculation. Half were not pasteurized and half were pasteurized in 85°C water for 16 minutes. All samples were stored at 4°C for up to 85 days and examined periodically. Post-packaging pasteurization eliminated microflora and significantly reduced the levels of three strains of *L. monocytogenes* on the surface and in the broth of the precooked beef chunks for 85 days of refriger-

ated storage as determined by direct plating procedures. All three strains of *L. monocytogenes* were recovered from the inoculated pasteurized beef using enrichment. Uninoculated chunks were positive for *Listeria* spp. and were primarily *L. welshimeri*. Without pasteurization, microflora reached high levels within 14 days of storage. The levels of all three strains of *L. monocytogenes* increased significantly during 7 days and remained unchanged for the remainder of the storage period.

Incidence and Persistence of *Listeria monocytogenes* on Vacuum-Packaged Primal Beef

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Listeria monocytogenes has emerged as one of the most important foodborne pathogens. It is widespread in nature and often associated with domesticated livestock. The incidence of *L. monocytogenes* in raw meats ranges from 4% to 92% of samples tested. Some sources of *L. monocytogenes* in these products have been identified, but exact sources are still somewhat unclear. The microbiological quality and safety of primal beef cuts originating from a single processing plant were assessed. Findings suggest that the distribution of *L. monocytogenes* in beef is not uniform and may be affected by type of cut.

Twenty-four random, vacuum-packaged cuts each from the round, loin, rib and chuck were obtained from a processing plant, transported directly to Auburn University Meat Laboratory and stored at −1°C. At 0, 25, 35, 49, 68, 82, 104 and 128 days of storage, three samples of each cut were evaluated. Microbiological samples were obtained by aseptically removing 100g (total) from six locations on the surface of each cut. Each sample included 200 cm^2 of surface area with 0.5 cm of underlying tissue. Each sample was mixed and a subsample (25g) was analyzed for the presence of *L. monocytogenes* using USDA methodology.

Cut samples analyzed at 0, 25 and 35 days of storage were repackaged, restored (0- and 25-day samples) at −1°C for a total storage time of 35 days, then placed at 4°C. After 7, 14 and 21 days of storage (4°C), samples were obtained and analyzed as described above.

A high incidence of *L. monocytogenes* was found on all cuts except chuckrolls. For total samples taken from −1°C storage, 62% (n=96) yielded *L. monocytogenes*, and 83%, 67%, 75% and 25% (n = 24) of rib, loin, round and chuck samples, respectively, yielded the pathogen. At each sampling period, chuckroll samples had the lowest incidence. For total samples from −1°C + 4°C storage, 50% (n = 72) contained *L. monocytogenes* and 61%, 44%, 72% and 22% (n = 18) of rib, loin, round and chuck samples, respectively, yielded the pathogen.

In both storage scenarios, the chuckroll consistently had the lowest incidence of *L. monocytogenes*. These data suggest that *L. monocytogenes* is not evenly distributed on beef cuts. The reasons for this may relate to type of cut and initial carcass contamination.