Tuesday, April 24, Juniper Room

9 a.m. Opening Remarks, Discussion of the Agenda, Review of Minutes from October Meeting, Committee Survey - Tracie Sheehan, Sara Lee Foods

9:15 a.m. Updates
Meat and Cancer Briefing – Randy Huffman, AMI
IARC and WCRF Preparation – Huffman
CDC Publication on O157 Declines – Huffman

10:15 a.m. Break

10:30 a.m. “Naturally Cured” Issue – Safety Considerations - Robin Peterson

11 a.m. Prepare for Joint AMI-FMI Meeting
Deli Best Practices – how can AMI assist retail?
Discuss Deli Slicer Research at UGA and MSU
Cloning Comments to FDA

12 p.m. Lunch (provided)

1 p.m. Joint AMI – FMI meeting followed by Joint Dinner (separate agenda)

Wednesday, April 25, Oak Room

7:30 a.m. Continental Breakfast

8 a.m. Wrap-up and Next Steps from Joint FMI Meeting

8:30 a.m. Avian Influenza Virus Inactivation Research – Tracie Sheehan

9:00 a.m. 2007-08 FSIS Dioxin Survey – Randy Huffman
O157 Sampling and Testing Challenges

9:30 a.m. Break

9:45 a.m. Report on AMIF Condensation Study - Mindy Brashears, Texas Tech University (via speakerphone)

10:30 a.m. AMIF Research Update: Newly Approved Projects
Ideas for Targeted projects
Priorities for 2008

11:30 a.m. New Business / Hot Topics
Clostridium difficile
Mycobacterium paratuberculosis Colloquium

12 p.m. Adjourn
AGENDA FOR JOINT AMI/FMI COMMITTEE MEETING

APRIL 24, 2007

1:00
• Meeting convenes in the Pomodoro Room

• Welcome and Introduction
  • Randy Huffman, AMI / Jill Hollingsworth, FMI
  • Tracie Sheehan, Sara Lee Corp., AMI Scientific Affairs Committee
  • Cas Tryba, Big Y Foods, FMI Food Protection Committee

1:15 – 1:45
• New York *Listeria* Project
  • Joe Corby, NYS Ag & Markets (conference call)

1:45 – 2:45
• RLM (Risk-Based *Lm* Sampling) – Tracie Sheehan, Sara Lee Corp.
  • USDA-FSIS environmental sampling protocol for *Lm* in RTE meat processing facilities
• *Lm* sampling at retail – Paul Marra, Wegmans
  • Uniform sampling protocol – NY State

2:45 – 3:15
• AMI/FMI *Listeria* Research Project
  • Randy and Jill

3:15 – 3:30 BREAK

3:30 – 4:30
• CDC Update – Olga Henao, PhD
  • 2006 FoodNet Data
  • *Clostridium difficile*: An emerging pathogen of concern

4:30 – 5:30
• Miscellaneous Topics and Updates
  • Cloning
    • Barbara Glenn, Biotechnology Industry Organization
    • Leah Wilkinson, ViaGen (conference call)

  • Carbon Monoxide – AMI update
  • Link between meat consumption and cancer - Randy
    • World Cancer Research Fund 2007 Report
    • Preparing a response and anticipated negative media coverage

6:00 p.m.
• Meet in lobby

6:30 p.m.
• Dinner at Taverna 100
• Sponsored by ConAgra (Paul Hall)
Committee Business
MEMORANDUM FOR AMI SCIENTIFIC AFFAIRS ADVISORY COMMITTEE

FROM: RANDY HUFFMAN, DANE BERNARD, TRACIE SHEEHAN

SUBJECT: MINUTES – OCTOBER 3, 2006 MEETING, HOLLYWOOD FL

COMMITTEE MEMBERS IN ATTENDANCE:

Dane Bernard, Keystone Foods
Wafa Birbari, Sara Lee Corp.
Peter Bodnaruk, Ecolab
Michael Bradley, Premium Standard Farms
John Butts, Land O’Frost
Bill Christenson, Ed Miniat Inc.
Rick Fahle, Fairbank Farms
Tim Freier, Cargill
Margaret Hardin, Boar’s Head Provisions
Kevin Ladwig, Johnsonville Sausage, Inc.

Phil Minerich, Hormel Foods Corp.
Alan Oser, Hatfield Quality Meats
Steve Quickert, Kraft / Oscar Mayer
Tracie Sheehan, Sara Lee Corp.
Dennis Stifler, Coleman Natural Meats
Scott Stillwell, Tyson Foods, Inc.
Haley Walls, Purac America
Patricia Wester, SGS
Kurt Westmoreland, Silliker, Inc

GUESTS (in order of attendance during the meeting):
Steve Larsen, National Pork Board
Mohammad Koohmaraie, USDA - ARS

AMI STAFF:
Jim Hodges
Randy Huffman
Skip Seward

The AMI Scientific Affairs Advisory Committee (SAC) met on October 3, 2006, in Hollywood Florida, the day prior to the Annual Meat Industry Research Conference, and the AMI Innovation Showcase. The materials from the meeting have been posted as a PDF file on www.meatami.com with “members only” security access. The file can be located under the “committee resources” section, under the Scientific Affairs section.

Chairman Dane Bernard opened the meeting with a welcome and an overview of the agenda, which was accepted. The minutes from the March 2006 SAC meeting were accepted as submitted.
**Lm Intervention updates**

*Status of FSIS approval of Benzoate/Propionate/Sorbate*

Skip Seward provided a status update on FSIS approval for the use of the antimycotics, sorbate, benzoate and propionate in RTE meat products. Industry requested the approval for use of low levels of these ingredients in RTE products for the purpose of controlling *Listeria* growth. Several research projects have demonstrated the effectiveness of these compounds as anti-Listerial agents, including recent AMIF-funded research conducted at the University of Wisconsin. The request was made through the FSIS New Technology Office, and AMI has provided data on efficacy and safety to the agency. On June 25, Doug Palo of FSIS –NTO provided a draft of waiver of regulations guidelines that could allow a company to proceed with in-plant testing. The draft waiver guidelines included requirements for establishments to provide protocol and procedures for the use, labeling, use-by or sell-by package dating, and validation and verification information concerning the use. Subsequently, FSIS posed 3 additional questions to AMI regarding the toxicity and potential for formation of compounds of “unknown” toxicity as well as potential adverse reactions that were indicated in a 1980 ARS study on sorbate in bacon. AMI along with Dr. Kathy Glass of University of Wisconsin, responded to these questions, and we are currently awaiting further guidance from FSIS –NTO.

*Status of FDA – FSIS approval of Bacteriophage*

Randy Huffman provided an overview of the recent food additive petition approval by FDA for the use of bacteriophage as a *Listeria* intervention in RTE foods including RTE meat and poultry products. AMI staff met with Intralytics CEO for a briefing on the company, its technology and their plans for making the product available. AMI staff has also met with a Salt Lake City based firm, Omnilytics to learn of their bacteriophage applications in the produce industry and interest in targeting *E. coli* O157 in cattle. That briefing led to an invitation for the company CEO of Omnilytics to speak at the MIRC. Both firms indicate that the technology has great promise; however, it seemed apparent to AMI staff that significant work on the consumer education front must be accomplished, and based upon the technical data provided to AMI; significantly more work is needed in the specific applications of *Listeria*-specific phage to RTE products as well as O157-specific phage in cattle. The SAC had significant discussion concerning the approval announcement and several firms pointed out the significant amount of customer and consumer feedback, mostly negative, that was received after the FDA announcement and subsequent media coverage.

*Octi-gon Update - Ecolab*

Peter Bodnaruk and Tim Freier provided a brief update on the status of the Octi-gon technology as an effective *Listeria* intervention in certain applications. The technology is based upon surface application determined by the surface area of the product. Organoleptic effects have been minimal and it can be treated as a processing aid.

*Retail Deli Task Force on Lm*

Huffman provided an update on the actions of the joint AMI FMI task force established after the March 2006 SAC meeting. The group has met via conference call and several actions have been taken. At the request of FMI members a sample blinding protocol was developed by Silliker and reviewed by legal counsel. This procedure appears to be acceptable to all parties. The group had discussions concerning sample site selection and FMI was to provide an alternative set of sampling locations than what was proposed. The release of the Draughon study at the Conference on Food Protection and the IAFP meeting in August was generally well-received as Dr. Draughon made a
point to emphasize how the *Lm* prevalence rates had declined when these data were compared to the previous NFPA – Gombas et al. study.

The SAC had significant discussion regarding the need to conduct the retail study, in light of the Draughon study results, as well as the on-going Cornell and NYS Ag and Markets retail deli survey. There was agreement that there continues to be a need for seeking solutions to drive prevalence levels down in retail deli processing areas, and the AMI SAC wants to serve as a technical resource to our retail partners. It was agreed that the Silliker project may still have merit and funding for the project should be pursued again through the AMIF research funding process. The following resolution was agreed upon.

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AMI SAC will continue to work jointly with the FMI technical and regulatory committee through the Joint Listeria Task Force to identify opportunities to work together on the challenge of Listeria control. The proposed retail deli research project should be revisited in light of the Draughon and the Cornell studies and AMI should continue to seek input from the retail community and FMI on how best to proceed with the proposed research protocol.
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**FSIS response to AMI comments on *Listeria* Risk Assessment**

Huffman provided a brief overview of the FSIS response to AMI comments submitted on the FSIS *Listeria* Risk Assessment. The response to comments is posted on the FSIS website, but generally the agency was dismissive of the AMI feedback and did not directly address several of the data submissions that were included in the Exponent review. It is unclear what purpose the risk assessment is serving at FSIS, other than the need to conduct a risk assessment to satisfy OMB requirements when a new rule is proposed. The committee suggested that AMI should determine if Exponent would have an interest in speaking in public forums regarding the work they did on behalf of AMIF and possibly publishing the FSIS *Listeria* risk assessment critique. AMI will work with Exponent to determine if there are possible avenues to make the review a more public document to ensure the review and suggestions are considered as part of future FSIS work in this area.

**NACMCF Report on *Campylobacter* Methods**

Wafa Birbari provided a presentation to the committee on the National Advisory Committee report on Campylobacter methodology. The PowerPoint has been included in the SAC briefing materials.

**NAS Dioxin Review**

Huffman provided an update on the release of the National Academy of Sciences review of the EPA Dioxin Reassessment. A detailed point by point critique and assessment of the charge questions posed to the NAS committee was provided in the SAC briefing materials. AMI has been working jointly with the Food Industry Dioxin Working group regarding the release of the report. The release generated very limited media interest at the time of the release. The NAS report is likely to lead to EPA going back to the drawing board on some aspects of the risk assessment, creating further delay in its finalization.
**IARC update and Discussion of Nitrite Alternative Research**

Andy Milkowski provided an update on the International Agency for Research on Cancer (IARC) report on ingested nitrites and nitrates. A full report was given during the preceding day by Jim Coughlin and Andy during the MIRC, and the Lancet publication related to the IARC decision were provided in the SAC briefing materials. The IARC decision that ingested nitrite, under conditions that result in endogenous nitrosation is probably carcinogenic in humans, could have significant impact on the future of nitrite as a food ingredient. In light of this potential challenge, Andy proposed to the group that consideration should be given to AMIF funding of curing alternatives research program. Andy provided two examples of recent research publications from the medical community related to myoglobin / muscle biochemistry. It was suggested that the meat science field may be able to draw upon work being done in the field of medical research. It was generally agreed that this may be an area worthy of pursuit. It was discussed that this type of research could generally fall within the realm of food safety research and may be an appropriate topic under the current AMIF research priorities. Andy agreed to develop a concept paper to more fully develop and outline what types of research may need to be pursued to yield tangible results.

**WCRF update and discussion of Nutrition and Health Roundtable**

Huffman and Jim Hodges led a discussion about the pending release of the World Cancer Research Fund report, due out in November 2007. It is expected that this report will result in negative messages relative to red and processed meat consumption. AMI has been working jointly with the International Meat Secretariat Human Health and Nutrition committee in preparation for this report for several years. One idea that has been proposed is the formation of a North American roundtable to discuss the science and identify leading experts in the field of meat and cancer research that are willing to discuss the benefits of meat in a balanced diet. AMI has been working closely with National Pork Board and National Cattlemen’s Beef Association on this concept. The SAC had good discussion on the issue and there was support for the following resolution:

> The SAC recognized the importance of the WCRF release to long-term consumer confidence in meat safety. The committee encouraged AMI staff to conduct a North American Roundtable on Meat and Cancer with counterparts in Beef and Pork commodity groups. Funding for such an endeavor should be sought from the AMI Executive committee as necessary.

**National Pork Board Take Care Program and Antibiotic Resistance**

Steve Larsen from the National Pork Board provided a presentation on the recently developed on-farm outreach program to producers to address the prudent use of antibiotics in swine production. A copy of the presentation is provided in the SAC briefing materials and companies seeking further information are encourage to contact Steve at the NPB.

**MDR –*Salmonella* update**

Freier provided an overview of his concerns related to the methods that FSIS is using for MDR *Salmonella* testing, and as part of the NACMCF, Tim states that the committee has been charged with evaluating these methods. The link between serotypes causing human disease, and those commonly found in various meat samples is not well established and attribution remains a major
challenge. Another primary concern is that the industry has no way of reliably controlling for specific *Salmonella* subtypes, since serotyping costs are significant (~$120/sample). No resolution was proposed during this discussion, but it was considered a topic that the SAC should keep close tabs on. There may be components of this issue that require research and could become part of the AMIF research priorities.

**Mycobacterium paratuberculosis update**

A PowerPoint presentation given by Mark Klaussen of the Canadian Beef Information Center during the 2005 Beef Safety Summit was provided in the SAC Briefing pdf as background. Freier provided an overview of the NACMCF review of MAP, including the frequency of the pathogen in various foods, the current testing methodologies, and how it is affected by processing techniques. He pointed out that the organism is very difficult to work with and detection times are very long. Using DNA based methods, MAP has been found in approximately 30% of pasteurized milk samples. MAP is the principle cause of Johne’s disease in cattle, and there is a hypothesis that is related to Chrone’s disease in humans, however, this relationship is still unclear. About 1 million people in the US are affected by Chrone’s disease.

**Update on CDC Publication on *E. coli* O157 Illness Decline**

AMI continues to collaborate with CDC representatives as they work to prepare a publication for Emerging and Infectious Diseases journal describing the *E. coli* O157 illness declines and the steps that industry has taken to control the pathogen. Huffman provided an update on the AMI contribution to the project. The committee suggested that AMI make certain that CDC describes the actions of industry in the proper light. It was pointed out that we should make certain that the publication does not simply state that product testing and diversion of product positives was the cause of some of the illness decline, but to stress that the actions that the industry had to take, as a result of the test and hold programs was what has led to enhanced safety of beef products. The emphasis should be on how testing led to the need for enhanced and improved control techniques, and not on the mere removal of certain positive products from the marketplace. It was agreed that these points will be made with CDC representatives.

Huffman also made the committee aware of a draft of a recently funded AMIF project at UW – FRI describing all food and non-food related outbreaks of *E. coli* O157 since it’s emergence in 1982. The draft report was provided in the SAC briefing pdf.

**Update on USDA –ARS research on *E. coli* O157**

Dr. Mohammad Koohmaraie joined the committee to provide a preliminary review of new data that has been collected at the US Meat Animal Research center. He reviewed data on *Salmonella* prevalence in the Multi-region survey that his research group has conducted over the last several years. He specifically shared preliminary data on the prevalence of non-O157 STEC and MDR *Salmonella* strains. Dr. Koohmaraie shared preliminary data showing that lairage at beef slaughter may be responsible for re-contamination of cattle arriving at packing plants. Strain typing of cattle isolates before arrival, and after arrival, indicate that the holding pens at slaughter are a potential contributing factor to hide contamination.
Transition of SAC Chairmanship

Dane Bernard announced to the SAC that Tracie Sheehan would be the next AMI SAC chair. Dane served in the capacity for approximately 2 years and his contributions were recognized. Tracie Sheehan thanked the group for their support and had a brief discussion of plans for the spring meeting of the SAC. No final decision was made. Once a date is established it will be communicated to the committee via email.

With no further business, the meeting was adjourned.

cc: J. Patrick Boyle
    Jim Hodges
Updates
<table>
<thead>
<tr>
<th>Time</th>
<th>Session</th>
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<td>10 a.m.</td>
<td>Introduction, Welcome and Statement of Purpose</td>
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| 10:15 a.m. | Caloric Intake, Exercise and Weight Control as Key Lifestyle Factors in Reducing Cancer Risk  
*David M. Klurfeld, Ph.D., National Program Leader, Human Nutrition, Agricultural Research Service, U.S. Department of Agriculture* |
| 11 a.m.    | Heterocyclic Amine Formation During Meat Cookery                        
*James Felton, Ph.D., Group Leader, Molecular Toxicology Group, Lawrence Livermore National Laboratory* |
| 12 – 1:15 p.m. | Lunch                                                                  |
| 1:15 p.m.  | Epidemiology and Genetic Interactions of CRC and other Cancer End Points Related to Meat Cooking Methods  
*Rashmi Sinha, Ph.D., Senior Investigator, Division of Cancer Epidemiology and Genetics, National Cancer Institute* |
| 2:15 p.m.  | A Safety Assessment of Nitrate / Nitrite Exposure from Food             
*James R. Coughlin, Ph.D., President, Coughlin & Associates* |
| 3 – 3:15 p.m. | Break                                                                  |
| 3:15 p.m.  | Conjugated Linoleic Acid and its Protective Role in Cancer              
*Martha Belury, Ph.D., R.D., Professor, Department of Human Nutrition, Ohio State University* |
| 4 p.m.     | Role of the Total Diet & Bioactive Food Components in Human Cancer - Putting it all Together  
*John Milner, Ph.D., Chief, Nutrition Science Research Group, National Cancer Institute* |
| 4:45 – 5:15 p.m. | Discussion, Wrap up and Adjourn                                  |
Draft Agenda - Day 2
Industry Only Roundtable
Meat and Cancer
March 7th 2007
Hogan and Hartson Conference Center
555 13th Street, NW
Washington, DC

8:00         Introduction, Statement of Purpose
8:15 – 10:00 Open discussion of Day 1 session
             What was learned?
             Key takeaway messages on the science.
             What are the primary gaps in knowledge?
             Expected conclusions from WCRF report
             What key scientific issues were not covered in Day 1?
10:00- 10:30 BREAK
10:30 – 12:00 Development of Draft Action Plan
             Work on the key science concepts that can inform the communication messages
             What are the underlying positive messages about meat in the diet that can be discussed in the context of the WCRF REPORT?

NOTE: AMI opinion is that this ½ day session should not be used as a communication strategy session. Rather the time should be strictly focused upon the key issues surrounding the science that will assist in development of sound messages. Others may have a different point of view, but we should discuss if so!

Attendees for Day 2: Industry Association staff, key industry technical member representatives, targeted industry consultants. Speakers from Day 1 are not invited, unless specifically representing one of the sponsoring associations as a consultant. No press, no open invitations, no open announcement of meeting to public.

*Please note no speakers have been invited.
“Naturally Cured” Issue – Safety
Natural and Organic Cured Meat Products: Regulatory, Manufacturing, Marketing, Quality and Safety Issues

Joseph Sebranek and James Bacus

Introduction

Natural and organic processed meats have been a very significant part of the explosive market growth that is occurring in natural and organic foods. Producers and processors have responded to consumer demand for foods perceived by many to be more healthy and wholesome than conventionally produced food products. To qualify as natural or organic, foods must be produced and processed in accordance with United States Department of Agriculture (USDA) regulations that define these products. In most cases, natural and organic foods very closely resemble conventional products and do not differ in the typical characteristics expected by consumers. However, in the case of processed meat products such as hams, bacon, frankfurters, bologna and others that are typically cured by addition of sodium nitrite, and sometimes sodium nitrate, the requirements for natural or organic marketing do not permit addition of nitrite or nitrate. Nitrite, whether added directly or derived from nitrate, is a unique, distinctive ingredient for which there is no substitute, consequently process and product changes are necessary to produce natural or organic processed meats that provide the properties expected of traditional cured meat products. These changes, combined with additional labeling requirements for these products, have resulted in a category of processed meats that is confusing, and perhaps even misleading, to consumers. Further, because of the key role that nitrite plays in cured meat quality and safety, quality and safety issues need to be carefully examined in light of the processing changes that are being introduced for manufacturing natural and organic processed meats.

Background

Growth in organic markets.

The annual growth in availability of natural and organic foods in the United States and around the world has been dramatic as producers and processors have responded to consumer demand for foods perceived to be “healthy” and “wholesome”, even though many of the health-related claims have been difficult to substantiate scientifically. Over the past 15-plus years, since 1990, organic food sales have increased by nearly 20% each year (Winter and Davis, 2006). Meat, poultry and seafood has been the fastest growing category of organic foods, increasing by 55.4% in 2005 alone (Mitchell, 2006; Organic Trade Association, 2006). While the organic and natural foods segment is still a relatively small part of the total food industry, comprising a 2.5% share in 2005, it is expected to increase to a 5–10% share in the near future (Nutrition Business Journal, 2006). Several studies have documented that consumer preferences for organic and natural foods are based on concerns about antibiotics, pesticides, hormones, genetic modifications in plants and animals, and chemical additives that consumers associate with conventionally produced foods (Bourn and Prescott, 2002; Dreezens et al., 2005; Sederer et al., 2005; Saher et al., 2006; Winter and Davis, 2006; Devcich et al., 2007). Consumers have expressed their preferences with a strong willingness to pay significant premiums for organic and natural foods. Premiums of 10–40% for organic foods over conventional products are common (Winter and Davis, 2006), but for meat and poultry, premiums may reach 200% (Bacus, 2006) or even more. In one such example, the average retail price for four brands of organic broilers in the Midwest during April and May, 2006 was $3.19/lb compared to $1.29/lb for conventionally produced broilers, a 247% difference (Husak, 2007). Prices for organic processed meats have been reported to range from $7.98/lb to $12.99/lb (Anon., 2005).

The large premiums that consumers are willing to pay for natural and organic foods have resulted in a rapid proliferation of new products and increased marketing by retailers. While in the past, the primary retailers of natural and organic foods were small cooperatives and health food stores, the success and rapid growth of retailers like Trader Joe’s Co. (over 200 stores), Whole Foods Market (181 stores), Wild Oats Market (110 stores) and Holiday Quality Food (23 stores) that feature natural and organic foods has resulted in many major supermarkets, most notably Wal-Mart, now offering these products for consumers (Petrak, 2005).
Definitions of natural and organic processed meats

The requirements that must be met for processed meats such as hams, bacon, frankfurters and bologna to qualify as natural or organic have resulted in unique and unusual approaches to the development of these products. This is because, while “natural” and “organic” are two separate and distinct categories of meat and poultry products in terms of USDA regulations and labels, neither of these product categories can be manufactured with added sodium (or potassium) nitrite or nitrate. Because nitrate and/or nitrite create distinctive, unique properties that characterize cured meat, and because there is no known substitute for these compounds, products manufactured to simulate cured meats but without added nitrite or nitrate, and without any other modifications, will be unattractive and atypical. However, the USDA permits the manufacture of uncured versions of typical cured meats according to the Code of Federal Regulations (9 CFR 319.2) (2006) which reads:

“Any product, such as frankfurters and corned beef, for which there is a standard in this part and to which nitrate or nitrite is permitted or required to be added, may be prepared without nitrate or nitrite and labeled with such standard name when immediately preceded with the term “Uncured” in the same size and style of lettering as the rest of such standard name: Provided, That the product is found by the Administrator to be similar in size, flavor, consistency and general appearance to such products as commonly prepared with nitrate and nitrite: And providing further, That labeling for such products complies with the provisions of 317.17 (C) of this subchapter”.

Thus, there is another category of processed meats, separate from “natural” and “organic”, and that category is “uncured”. The definitions of natural and organic require that “Uncured” be included for products labeled with a standardized cured product name (i.e., bacon), but it is important to note that not all products labeled “Uncured” are natural or organic.

Processed meats that are labeled “natural” must comply with the definition of the term provided by the USDA Food Standards and Labeling Policy Book (USDA, 2005). This definition requires that a natural product …

“…does not contain any artificial flavor or flavoring, coloring ingredient, or chemical preservative (as defined in 21 CFR 101.22), or any other artificial or synthetic ingredient; and the product and its ingredients are not more than minimally processed.”

The term “minimally processed” includes “…traditional processes used to make food edible or to preserve it or to make it safe for human consumption, e.g., smoking, roasting, freezing, drying, and fermenting, or those physical processes which do not fundamentally alter the raw product…, e.g., grinding meat…” (USDA, 2005).

The definition of natural has not been without controversy. For example, in the 2005 edition of the USDA Food Standards and Labeling Policy Book, a note was added indicating that sugar, sodium lactate (from a corn source) and natural flavorings from oleoresins or extractives are acceptable for “all natural” claims. However, because lactate is widely recognized as an antimicrobial ingredient, such use may conflict with the “no chemical preservatives” requirement for labeling of a product as natural. This was the basis for a petition submitted to the USDA in October, 2006 after which the Agency removed lactate from the guidance statement provided for natural claims. The USDA will, however, consider use of lactate for natural foods on a case-by-case basis for applications where the ingredient may function as a flavoring rather than a preservative. Further, the Agency is currently planning to initiate new rulemaking processes in the near future for the use of the term “natural” to clarify these uses as well as the use of natural claims relative to livestock production practices (O’Connor, 2006). Currently, natural claims do not include consideration of animal production practices, and all fresh meat qualifies as natural. There is a significant amount of interest by both producers and consumers in the establishment of standards for meat animal production systems that would satisfy the perceptions consumers currently hold regarding natural foods. Consequently, it is likely that the labeling of processed meats as natural, which currently means minimally processed with no artificial ingredients or preservatives, will be more broadly defined in the near future. As of this writing, the USDA is soliciting comments on this issue until March 5, 2007 after which the rulemaking process for natural claims is expected to begin.

Products labeled as organic are much better defined and controlled than those labeled with natural claims because organic products are governed by the USDA Organic Foods Production Act (OFPA), first passed in 1990 as part of the 1990 Farm Bill (Winter and Davis, 2006). The OFPA created a National Organic Standards Board, which established a National List of Allowed and Prohibited Substances, and developed National Organic Program Standards. The standards, implemented in 2002, specify methods, practices and substances that may be used for production, processing and handling of organic foods. This means that products and ingredients used for organic foods must be certified as organic by a USDA-certified inspector. Meat, for example, must be raised using organic management and come from a certified farm. Ingredients used for processed products are clearly defined as permitted or prohibited in the OFPA National List. Organic products may be labeled in four different ways: 1) “100 % organic”, which must contain only organically produced ingredients; 2) “organic”, which must contain at least 95% organically produced ingredients; or 3) “made with organic ingredients”, which must have at least 70% organic ingredients. Products with less than 70% organic ingredients might be considered as the 4th labeling option but are not allowed to be labeled as organic and are permitted only to list those ingredients that are organic on the information (ingredients) panel of the label. Those products that qualify for the "organic"
Definitions of cured and uncured processed meats

The term “cured” relative to processed meats is universally understood to mean the addition of nitrite or nitrate with salt and other ingredients to meat for improved preservation (Pegg and Shahidi, 2000). While several ingredients including sugar, spices, phosphates and other ingredients are typically included in cured meats, it is the addition of nitrate/nitrite in one form or another that results in the distinctive characteristics of cured meat (Casens, 1990). The typical color, flavor, shelf life and safety of ham, bacon, frankfurters, bologna and other cured products are so widely recognized by consumers that these product names are considered “standardized” and “traditional” by the USDA for product labeling and therefore do not require any further clarification to communicate the expected product properties to consumers. On the other hand, products that are similar but made without nitrite or nitrate, must be clearly labeled as “Uncured” as described earlier. This is because “uncured” versions of standardized products like ham, bacon, frankfurters and bologna are significantly different from the traditional products that they emulate. At the same time, there are a number of processed meats that are traditionally manufactured without nitrite or nitrate, and that are not labeled as uncured because the standardized product name effectively communicates that the product is not cured. Fresh sausage, such as pork sausage, for example, is not labeled as “uncured” because these products are standardized, traditional and the common name is clearly understood.

The advent of natural and organic processed meat products, both of which prohibit direct addition of nitrite or nitrate, but that also resemble traditional cured meat, has made it necessary to require “uncured” as part of the traditional product name. However, because current meat processing technology has developed means by which nitrate and nitrite can be indirectly added to these products to achieve very typical cured meat properties, the labeling designations for these products as “uncured” is sometimes confusing and is technically inaccurate. Further, because the indirect addition of nitrate and nitrite to natural and organic processed meats has not been thoroughly investigated in terms of nitrite chemistry and subsequent product properties, a number of important questions concerning quality and safety remain to be answered.

Conventional Cured Meat Ingredients and Processes

Conventionally-cured meat products are characterized by the addition of nitrate and/or nitrite. While other ingredients, particularly sodium chloride, are essential parts of typical cured meat formulations, it is the nitrate/nitrite that provides the distinctive properties that are common to all cured meat products. The role of nitrate/nitrite is so commonly understood in the meat industry that the term “cure” is used as both a noun and a verb, meaning either nitrate/nitrite as chemical entities, or the addition of these ingredients to meat, respectively.

Nitrate

Numerous reviews of the history of meat curing have suggested that meat curing originally developed from the use of salt contaminated with sodium or potassium nitrate (Binkerd and Kolari, 1975; Sebranek, 1979; Pierson and Smoot, 1982; National Academy of Sciences, 1982; Cassens, 1990; Pegg and Shahidi, 2000).

While it is not clear when saltpeter (potassium nitrate) was first recognized as a curing agent, it is clear that nitrate, either as saltpeter or as a contaminant of sodium chloride, was used to cure meat for centuries before research chemists began to unravel the chemistry of meat curing. In the late 1800’s, it was discovered that nitrate was converted to nitrite by nitrate-reducing bacteria, and that nitrite was the true curing agent. Further research in the early 1900’s established the appropriate concentrations of nitrate and nitrite to be used in cured meat, and resulted in authorization by the USDA in 1925 of the use of sodium nitrite.

The following 45 years brought a gradual shift from nitrate to nitrite as the primary curing agent for cured meats as the advantages of faster curing time for increased production capacity became more important, and as nitrite chemistry became better understood. By the early 1970’s, relatively little nitrate was being used for cured meats (Binkerd and Kolari, 1975). The late 1960’s and early 1970’s also brought a watershed event for the cured meat industry when it became obvious that nitrite could result in the formation of carcinogenic n-nitrosamines in cured meat. Subsequent research demonstrated that a significant factor in nitrosamine formation was residual nitrite concentration, and consequently, nitrite was eliminated from most curing processes to achieve better control over residual nitrite concentrations (Pegg and Shahidi, 2000). Today, nitrate is seldom used and then only in a few specialty products such as dry cured hams and dry sausage where long, slow curing processes necessitate a long-term reservoir of nitrite that can be slowly released over the course of the process.

Nitrite

The chemistry of nitrite in cured meat is a fascinating, sometimes frustrating mixture of interactive chemical reactions involving several different reactants and affected by several different environmental factors. Nitrite is a highly reactive compound that can function as an oxidizing, reducing or a nitrosating agent, and can be converted to a variety of related compounds in meat including nitrate, nitrous acid and nitric oxide. To further complicate the understanding of nitrite chemistry, it has become clear that the formation of nitric oxide (NO) from nitrite is a necessary prerequisite for many meat curing reactions (Møller and Skibsted, 2002). Fortunately, fundamental research on nitric oxide has become one of the most active research areas in biology because nitric oxide has been found to play crucial roles in several physiological functions in living organisms. For example, in skeletal muscle, nitric oxide appears to interact with proteins such as the ryanodine receptor-calcium release channel and regulates muscle functions including excitation-contraction coupling, blood flow, respiration and glucose homeostasis (Stamler and Meissner, 2001). Nitric oxide also plays a role in blood pressure control and immunity (CAST, 2001).
The most effective way to consider nitrite chemistry in cured meat is to consider the practical effects of the addition of nitrite to meat. The first and most obvious effect is that of cured color development. Close examination of the chemical reactions likely to be involved with color development immediately make it obvious that the chemistry of nitrite in meat is a phenomenally complex event. For example, nitrite (NO$_2^-$) does not act directly as a nitrosylating (transfer of nitric oxide) agent in meat but first forms one or more of several intermediates that are highly reactive. For example, it is recognized that HNO$_2$ (nitrous acid) is formed from nitrite under acidic conditions (Fox and Thomson, 1963; Pegg and Shahidi, 2000) such as that in postmortem muscle. Further, it is believed that a principal reactive species, N$_2$O$_3$ (dinitrogen trioxide) (Fox and Thompson, 1963; Honikel, 2004) is formed from nitrous acid and will subsequently form NO (nitric oxide) or will react with other substrates in a meat mixture.

$$\text{NO}_2^- + \text{H}^+ \leftrightarrow \text{HNO}_2$$

$$2 \text{HNO}_2 \leftrightarrow \text{N}_2\text{O}_3 + \text{H}_2\text{O}$$

$$\text{N}_2\text{O}_3 \leftrightarrow \text{NO} + \text{NO}_2$$

One of the likely substrates for N$_2$O$_3$ in meat is a reductant such as ascorbate (H-ASC) or erythorbate which then yields nitric oxide (NO) (Fox and Thomson, 1963; Møller and Skibsted, 2002), thus providing another source of nitric oxide in cured meat.

$$\text{N}_2\text{O}_3 + 2 \text{H–ASC} \leftrightarrow 2 \text{dehydro-ASC} + \text{H}_2\text{O} + 2 \text{NO}$$

The NO that is formed will react with the iron of both myoglobin (MbFe$^{3+}$) and metmyoglobin (M MbFe$^{4+}$) to form cured meat pigments and cured color. These reactions demonstrate two of the most important factors governing nitrite reactions in conventionally cured meat products, namely pH and the presence or absence of various reductants. However, several other nitrite reactions are involved in cured meat chemistry and contribute to nitric oxide production. For example, when nitrite is added to comminuted meat, the meat quickly turns brown because nitric acts as a strong heme pigment oxidant and is, in turn, reduced to nitric oxide.

$$\text{MbFe}^{4+} + \text{NO}_2^- \rightarrow \text{M MbFe}^{3+} + \text{NO} + \text{OH}^-$$

The NO reacts with metmyoglobin, and subsequent reduction reactions convert the oxidized heme to reduced nitric oxide myoglobin for the typical cured color following cooking.

$$\text{M MbFe}^{3+} + \text{NO} \rightarrow \text{M MbFe}^{3+} \rightarrow \text{reductant} \rightarrow \text{MbFe}^{4+}$$

To further complicate the system, all cured meats include sodium chloride in varying concentrations, and nitrite, as nitrous acid, will react with the chloride ion to form nitrosyl chloride, which is a more reactive nitrosylating agent than N$_2$O$_3$ (Sebranek and Fox, 1985, 1991; Fox et al., 1994; Møller and Skibsted, 2002).

$$\text{HNO}_2 + \text{H}^+ + \text{Cl}^- \leftrightarrow \text{NOCl} + \text{H}_2\text{O}$$

Consequently, chloride ions accelerate color development in cured meat.

Further, nitrite can also react with sulfhydryl groups on proteins to release nitric oxide in an oxidation–reduction reaction that results in a disulfide being formed (Pegg and Shahidi, 2000).

$$2 \text{RSH} + 2\text{HNO}_2 \rightarrow \text{RS-SR} + 2\text{NO} + 2\text{H}_2\text{O}$$

In addition to the above reactions of nitrite in meat, all of which affect the rate and/or extent of cured color development, nitrite plays a key role in cured meat as a bacteriostatic and bacteriocidal agent. Nitrite is strongly inhibitory of anaerobic bacteria, most importantly Clostridium botulinum, and contributes to control of other microorganisms such as Listeria monocytogenes. The effects of nitrite and the likely inhibitory mechanisms differ in different bacterial species (Tompkin, 2005). Nitrite is not generally considered to be effective for control of Gram-negative enteric pathogens such as Salmonella and Escherichia coli (Tompkin, 2005). However, Pichner et al. (2006) reported that E. coli survived longer and reached higher counts on salami without nitrite than on salami with added nitrite. The effectiveness of nitrite as an antibotulinal agent is dependent on several environmental factors including pH, sodium chloride concentration, reductants present and iron content among others (Tompkin, 2005). While the means by which nitrite achieves microbial inhibition is not clear and many mechanisms have been proposed, all of the factors that impact nitrite inhibitory effects are also important to the known reactions that generate nitric oxide for cured color. Consequently, it is likely that the reaction sequences involving nitric oxide and color development are also important players in the antimicrobial role of nitrite in cured meat. For example, some researchers have suggested that nitrous acid (HNO$_3$) and/or nitric oxide (NO) may be responsible for the inhibitory effects of nitrite while others have investigated several as-yet unidentified inhibitory substances (Tompkin, 2005). Reaction of nitric oxide with iron-sulfur enzymes of anaerobic bacteria has been reported to reduce the germination of these bacteria (Payne, et al., 1990). Because it appears that nitrite reactivity is key to microbial inhibition (one indicator of this is the strong dependence on pH), there has been some question whether ingoing or residual nitrite is most critical to antimicrobial effects. The USDA regulations that require specific minimum ingoing nitrite concentration of 120 ppm for cured products that are refrigerated (USDA, 1995) implies that ingoing levels of nitrite are critical. However, it may be that ingoing nitrite is important because ingoing nitrite affects the subsequent residual nitrite. Tompkin (2005) concluded that residual nitrite at the time of product temperature abuse is critical to antibotulinal effects and that depletion of residual nitrite during product storage will reach some point at which the inhibitory effects are also depleted. At the same time, it is interesting to note that residual nitrite in retail commercial cured meat products has declined by 80% from the 1970’s to a range of 1 to 16 ppm in 1997 (Cassens, 1997b) without any obvious effects on product safety.
The reaction sequences of nitrite and nitric oxide probably also play a key role in the strong antioxidant function of nitrite in cured meat, because proposed mechanisms for the antioxidant effect of nitrite include reaction with heme proteins and metals, including free iron, and formation of nitroso- and nitrosyl-compounds that have antioxidant properties (Pegg and Shahidi, 2000). It is likely that these proposed mechanisms are dependent upon many of the same initial reactions of nitrite that form nitric oxide for cured color. Thus, the affinity of nitric oxide for iron appears to play a role in several of the functions of nitrite in cured meat. The nitric oxide-heme complex contributes color, nitric oxide reaction with iron-sulfur enzymes may be important to inhibition of anaerobic bacteria, and nitric oxide combination with free iron and heme iron in meat reduces the catalytic role of iron in lipid oxidation.

Nitrite is also responsible for the production of characteristic cured meat flavor, although this is probably the least well understood aspect of nitrite chemistry (Pegg and Shahidi, 2000). It is easy to distinguish cooked, cured ham from fresh roast pork on the basis of flavor but the chemical identity of distinguishing flavor components in cured meat has eluded numerous researchers. Some of the flavor difference may be due to the suppression of lipid oxidation by nitrite but other antioxidants do not produce cured meat flavor. Pegg and Shahidi (2000) listed 138 volatile compounds identified in nitrite-cured ham. Some of the volatiles listed include nitrogen- or nitrogen/oxygen-containing compounds but there have been no suggested mechanisms that directly link nitrite or nitric oxide reactions to compounds identified as flavor components. If nitrite does, in fact, form volatile flavor factors, this would represent yet another reaction product of nitrite in cured meat.

In addition to the nitrite reactions which result in cured meat color, microbial inhibition, antioxidant effects and flavor, it has been demonstrated that addition of nitrite to meat results in formation of nitrate and nitrogen gas as well as reaction with carbohydrates and lipids (Pegg and Shahidi, 2000; Honikel, 2004).

Further, there are a number of curing accelerators that are commonly used to increase the rate of nitrite-to-nitric oxide conversion. These include acidulants such as glucono delta lactone (GDL), sodium acid pyrophosphate (SAPP) citric acid, sodium citrate and fumaric acid, and reductants such as ascorbic acid/sodium ascorbate and erythorbic acid/sodium erythorbate.

The effects of the acidulants and reductants on nitrite reactions are quite dramatic because both strongly affect the initial reduction of nitrite to nitric oxide as shown earlier. A pH decrease of 0.2 pH units, for example, is sufficient to double the rate of nitric oxide formation (Fox, 1974). Both acidulants and reductants result in significantly less residual nitrite in cured meat, an important contribution relative to the potential for nitrosamine formation associated with high residual nitrite concentrations.

The point of this condensed review of nitrite chemistry and the functions of nitrite in cured meat is not to reiterate what is commonly known about nitrite but rather to emphasize the highly reactive, complex nature of nitrite-meat mixtures. Because nitrite, particularly as nitric oxide, so readily reacts with a wide variety of substrates, reaction kinetics may be an important determinant of how nitrite is proportioned among the wide array of competitive substrates and reaction products. A slow formation of nitrite (such as from nitrate) in meat might be significantly different in terms of nitrite reaction products than the direct, one-time addition of a full load of nitrite. If, for example, the fastest-reacting substrates consumed a greater share of the nitrite during slow nitrite formation than in the case where nitrite is added directly, then the end products of the more reactive substrates might achieve a greater final concentration.

Past and current safety issues associated with nitrite
Issues that have been raised concerning the safety of using nitrate and nitrite for curing meat have included chemical toxicity, formation of carcinogens in food or after ingestion, and reproductive and developmental toxicity. None of these issues represent relevant concerns for nitrate or nitrite in light of the current levels of use in processed meats. While nitrite is recognized as a potentially toxic compound, and there have been cases where nitrite was mistakenly substituted for other compounds in food or drink at concentrations great enough to induce toxicity symptoms, the normally controlled use of nitrite in processed meats does not represent a toxicity risk under normal circumstances.

However, the issue of carcinogenic nitrosamines formed from nitrite in cured meat was a very serious concern in the 1970’s, and very nearly resulted in elimination of nitrite as a curing agent. Fortunately, changes in manufacturing practices and reduced levels of nitrite used in curing solved the problem of nitrosamine formation in cured meat. Yet, a low level concern about nitrite has lingered, and in the 1990’s a series of epidemiological studies reported that consumption of cured meat was related to childhood leukemia and brain cancer (Preston-Martin and Lijinsky, 1994; Sarasua and Savitz, 1994; Peters et al., 1994; Preston-Martin et al., 1996). Further, in 1998, nitrite was proposed to be classified as a developmental/reproductive toxicant under California’s Proposition 65 (Safe Drinking Water and Toxic Enforcement Act). Fortunately, both issues (nitrite as a carcinogen and as a developmental/reproductive toxicant) have been largely resolved by subsequent studies and careful scientific review of the available data (Milkowski, 2006).

The issue of ingested nitrate and nitrite first arose in the 1970’s when it was recognized that carcinogenic nitrosamines could be formed in the stomach following ingestion of nitrite. Subsequent work has shown that less than 5% of the nitrite and nitrate typically ingested comes from cured meat, the rest coming from vegetables and saliva (Cassens, 1997a; Archer, 2002; Milkowski, 2006). Nevertheless, in 2006, the International Agency for Research on Cancer (IARC) concluded that “Ingested nitrate or nitrite under conditions that result in endogenous nitrosation is probably carcinogenic to humans” (Coughlin, 2006). While the IARC report is still a work in progress, the conclusions are likely to ramp up questions and concerns about nitrite as a food additive. In light of the
anticipated challenges to nitrite in cured meat, it is imperative that as much information as possible is developed for all processed meat applications where nitrite and/or nitrate have a role.

Current U.S. regulations on nitrite and nitrate

Current regulations on the use of nitrite and nitrate in the United States vary depending on the method of curing used and the product that is cured. For comminuted products, the maximum ingoing concentration of sodium or potassium nitrite is 156 parts per million (ppm) or 0.25 oz per 100 lbs (7 g/45.4 kg), based on the green weight of the meat block (USDA, 1995). Maximum ingoing sodium or potassium nitrate for comminuted products is 1718 ppm. Sodium and potassium nitrite and nitrate are limited to the same quantity despite the greater molecular weight of the potassium salts. This means that the potassium salt contains less nitrite or nitrate than the equivalent weight of the sodium salt. For immersion cured, and massaged or pumped products, maximum ingoing concentration of sodium or potassium nitrite and nitrate concentrations are 200 and 700 ppm of nitrite and nitrate, respectively, again based on the green weight of the meat block. Dry cured products are limited to 625 ppm and 2187 ppm of nitrite and nitrate, respectively. If nitrite and nitrate are both used for a single product, the ingoing limits remain the same for each but the combination must not result in more than 200 ppm of analytically measured nitrite, calculated as sodium nitrite in the finished product.

Bacon is an exception to the general limits for using curing agents because of the potential for nitrosamine formation. For pumped and/or massaged bacon without the skin, 120 ppm of sodium nitrite or 148 ppm of potassium nitrite is required along with 550 ppm of sodium ascorbate or sodium erythorbate, which is also required. It is important to note that this is a specifically required amount whereas other nitrite limits are maximum quantities. To accommodate variation in pumping procedures and brine drainage from pumped products, the regulations for pumped and/or massaged bacon permit ± 20% variation from the target concentrations at the time of injecting or massaging. For example, sodium nitrite concentrations within the range of 96–144 ppm are acceptable. Nitrate is not permitted for any bacon curing method. There are two exceptions to these regulations for pumped and/or massaged bacon: first, 100 ppm of sodium nitrite (or 123 ppm of potassium nitrite) with an “appropriate partial quality control program” is permitted and, second, 40–80 ppm of sodium nitrite or 49–99 ppm of potassium nitrite is permitted if sugar and a lactic acid starter culture are included. Immersion cured bacon is limited to 120 ppm of sodium nitrite or 148 ppm of potassium nitrite while dry cured bacon is limited to 200 ppm or 246 ppm, respectively. For bellies cured with the skin on, the nitrite and reductant concentrations must be reduced by 10%, based on the assumption that skin comprises approximately 10% of the belly weight.

It is important to note that the regulations also require a minimum of 120 ppm of ingoing nitrite for all cured “Keep Refrigerated” products “unless safety is assured by some other preservation process, such as thermal processing, pH or moisture control.” The establishment of minimum ingoing nitrite concentration is considered critical to subsequent product safety. This is a significant consideration for natural and organic cured meat products.

On the other hand, for cured products that are processed to ensure shelf stability (may be stored at room temperature), there is no minimum ingoing nitrite level. The USDA Processing Inspector’s Calculations Handbook (USDA, 1995) suggests that, for shelf-stable products, “…40 ppm nitrite is useful in that it has some preservative effect. This amount has also been shown to be sufficient for color-fixing purposes…”

The curing accelerators permitted for use with nitrite are also restricted. Fumaric acid, for example, is limited to 650 ppm and only in cured, comminuted meat and poultry products, while GDL and SAPP are permitted at 5000 ppm only in cured comminuted meat products on a finished product basis. Ascorbic acid or sodium citrate may replace up to half of either form of the ascorbate/erythorbate reductants (USDA, 1995), but may not be used without the reductants.

Ingredients Used For Natural and Organic Cured Meats

Because of the negative perceptions of nitrite cured meat held by some consumers, the “uncured” natural and organic versions of typical cured meats have enjoyed widespread market acceptance. A survey of 56 commercial “uncured” meat products including bacon, ham, frankfurters, bologna, braunschweiger, salami, Polish sausage, Andouille sausage and snack sticks showed that most of these products demonstrated typical cured meat color and appearance (Sindelar, 2006b). A review of product ingredients statements showed that 38 products included sea salt, 33 listed evaporated cane juice, raw sugar or turbinado sugar, 19 included a lactic acid starter culture, 17 had natural spices or natural flavorings, 14 added honey and 11 included celery juice or celery juice concentrate. Interestingly enough, at the time of this survey (October–November, 2005), 16 of the products included lactate, which was recently removed from the USDA Food Standards and Labeling Policy Book guidance statement for ingredients acceptable for natural claims. Beets or other natural coloring agents are not permitted in natural products since these ingredients are viewed by the USDA as artificially coloring the meat product.

Analyses of samples of 4 selected commercial brands each of natural or organic bacon, hams and frankfurters showed that all samples except one sample of bacon contained residual nitrite at concentrations ranging from 0.9 ppm to 9.2 ppm. Residual nitrate was found in all products at concentrations of 6.8 ppm to 44.4 ppm (Sindelar, 2006a). Residual nitrite was lower in most of the natural or organic products at the time of sampling than in comparable commercial products made with the conventional addition of nitrite. Other cured meat properties including instrumental color, cured pigment concentration, lipid oxidation and sensory properties were, in general, similar for the natural or organic products.
relative to the conventionally cured products, but greater variation in the natural and organic products was obvious. Most notable were the low color values, low cured pigment content and low human sensory scores for those products that contained little or no residual nitrite. It is important to note that because these were commercial products selected at retail, the time of manufacture and storage history of each was unknown. Nevertheless, these results suggest that: 1) there is wide variation among the natural and organic processed meats that simulate conventionally cured products, and 2) a large majority of natural and organic processed meats demonstrate typical cured meat properties, including cured color, flavor and significant concentrations of residual nitrite and nitrate. Thus, it is clear that nitrite and nitrate are being introduced to most of these products indirectly as components of other ingredients.

Unique ingredients in natural and organic processed meats

The most common ingredient observed in review of the product labels of natural and organic processed meats was sea salt. Sea salt is derived directly from evaporation of sea water, unrefined without addition of free-flow additives and retains the natural trace minerals characteristic of the source (Kuhnlein, 1980; Heinerman and Anderson, 2001). Several varieties of sea salt are available and differ depending on the geographical origin of the water used and the mineral content (Saltworks, 2006). While sea salt has been suggested as a likely source of nitrate, limited analytical information suggested that the nitrate content of sea salt is relatively low. Herrador et al. (2005) reported that Mediterranean sea salt contained 1.1 ppm of nitrate and 1.2 ppm of nitrite. Cantoni et al. (1978) analyzed 10 samples each of 3 grades of sea salt and found nitrate and nitrite concentrations of 0.3–1.7 ppm and 0–0.45 ppm, respectively. As a GRAS substance, salt incorporated in food must comply with the Food Chemicals Codex tolerances for purity. Solar-evaporated sea salt must be at least 97.5% sodium chloride with specific limits on calcium/magnesium, arsenic and heavy metals content (Food Chemicals Codex, 2003).

The second most common ingredient observed in natural and organic processed meat ingredient lists was raw sugar, most often shown as turbinado sugar. Turbinado sugar is a raw sugar obtained from evaporation of sugar cane juice followed by centrifugation to remove surface molasses. Remaining molasses gives turbinado sugar a light brown color and flavor similar to brown sugar. While it seems possible that raw sugar could include nitrate, there appears to be no evidence of significant nitrate or nitrite concentrations in raw sugar.

Natural flavorings or spices, and celery juice or celery juice concentrate were frequently listed as ingredients, and because these are plant/vegetable products, the potential contribution of nitrate from these sources is very significant. Vegetables are well-known as a source of nitrate with concentrations as high as 1500 ppm to 2800 ppm (National Academy of Sciences, 1981) in celery, lettuce and beets. Vegetable juices and vegetable powders are commercially available and may be used as ingredients in natural and organic foods. Analysis of some commercially available vegetable juices showed that carrot, celery, beet and spinach juice contained 171, 2114, 2273 and 3227 ppm of nitrate, respectively (Sebranek, 2006). After 10 days of storage at room temperature, nitrate levels in these juices declined by 14–22%. Nitrite was not detected initially but concentrations of 128–189 ppm of nitrite were found after 10 days at room temperature, probably resulting from bacterial reduction of nitrate. Analysis of commercial celery juice powder indicated a nitrate content on the order of 27,500 ppm or about 2.75%, reflecting the increased concentration following drying (Sindelar, 2006a). Clearly, vegetable products offer the greatest potential to introduce natural sources of nitrate into processed meats. Juices and powders have advantages in supplying nitrate in concentrated form. Celery juice and celery powder appear to be highly compatible with processed meat products because celery has very little vegetable pigment (as opposed to beets, for example) and a mild flavor profile similar to raw celery that does not detract greatly from finished product flavor. Further, these vegetable products may be listed as natural flavoring on meat product labels.

A critical ingredient for processed meats with natural nitrate sources is a nitrate-reducing bacterial culture, if typical cured meat properties are the final objective. The necessity of bacterial reduction of nitrate to nitrite for meat curing was discovered in the 1890’s (Pegg and Shahidi, 2000) and nitrate reducing cultures have been commercially available for several years. Most applications of these cultures have been for dry sausage, where a long-term reservoir of nitrite during drying is desirable and where subtle flavor contributions from the culture are considered important (Olesen et al., 2004). The lactic acid starter cultures used for fermented sausage, primarily Lactobacillus plantarum and Pediococcus acidilactici, do not reduce nitrate. However, cultures of coagulase negative cocci such as Kocuria (formerly Micrococcus) varians, Staphylococcus xylosus, Staphylococcus carnosus and others will reduce nitrate to nitrite. These organisms can achieve nitrate reduction at 15–20°C but are much more effective at temperatures over 30°C (Casaburi et al., 2005). The typical recommended holding temperature for commercial nitrate reducing cultures is 38–42°C to minimize the time necessary for adequate nitrite formation. Recent research has documented that time is a critical parameter in the development of typical cured meat properties from natural sources of nitrate. Sindelar (2006a) reported that a holding time at 38°C was more critical than the amount of naturally-added nitrate for development of cured meat properties in small diameter cooked sausage (similar to frankfurters) and hams. Time appeared to be more critical for the small diameter cooked sausage that reached an internal temperature of 38°C quickly than for the large diameter hams where internal temperature increased to 38°C more slowly.

Sindelar et al. (2007a) also evaluated several quality characteristics of small diameter cooked sausages manufactured with starter culture and either 0.2 or 0.4% celery juice powder, each held at 38°C for either 30 or 120 minutes. The products were evaluated during 90 days of refrigerated, vacuum-packaged storage and compared with conventionally processed products manufactured at the
same time with added sodium nitrite. Color measurements (Hunt-
er $a^*$ values, reflectance ratios, cured pigment concentrations) indi-
cated that treatments with short incubation time resulted in less
cured color/redness than the nitrite-cured control although this
difference was not always significant. Cured color/redness of the
product made with the longer incubation time was, in general,
comparable to the nitrite-cured control. Residual nitrite following
incubation was dramatically different with 5.6 and 7.7 ppm found
for the 0.2% and 0.4% celery powder levels, respectively, after 30
minutes of holding time, but 24.5 ppm and 46.0 ppm were ob-
served after 120 minutes. No differences were noted for lipid ox-
idation between any of the treatments and the control. The nitrite-
cured control, in general, received the highest sensory scores al-
though differences were not significant for all sensory properties.

A similar experiment with hams (Sindelar et al., 2007b) was
conducted using either 0.2 or 0.35% celery powder and incubation
time of 0 or 120 minutes. The treatment with no incubation time
was included because the extended thermal process (3 hours, 35
minutes) used for hams relative to small diameter frankfurter-style
sausage was expected to result in adequate nitrate reduction by the
culture. Results showed that there were no treatment differences in
objective color measurements or cured pigment concentrations for
the hams, and all product treatments were similar in color proper-
ties to the nitrite-cured control. Residual nitrite, following the 120
minute incubation for the 0.2 and 0.35% celery juice powder ad-
ditions, was 19.5 and 36.1 ppm, respectively. The residual nitrite
was significantly less for the hams with celery juice powder (21.0–
36.0 ppm at day 0; 7.2–21.3 ppm after 90 days) relative to the
nitrite-cured control (63.4 ppm at day 0; 34.1 ppm after 90 days).
However, residual nitrite was greater in hams with a greater
amount of added celery juice (27.7–36.0 ppm from 0.35% celery
powder vs. 19.3–21.0 ppm from 0.20% celery powder at day 0
compared with 11.7–21.3 ppm vs. 7.2–8.8 ppm, respectively, for
each after 90 days). Sensory panel evaluations indicated that the
higher amount of celery powder (0.35%) resulted in greater vege-
table aroma and flavor with less ham aroma and flavor. The treat-
ments with a lower level of celery powder (0.2%) were similar to
the nitrite-cured control for all sensory properties evaluated.

The authors concluded that the celery juice powder/starter cul-
ture treatment was an effective alternative to the direct addition of
sodium nitrite to small-diameter, frankfurter-style cured sausage
but that incubation time at 38°C is an important factor for pro-
duct quality. The celery juice powder/starter culture treatment was
also effective for hams but in this case the amount of celery juice
powder proved to be more critical. For large diameter products
such as hams, it appears that the slow temperature increase that is
part of a typical thermal process may provide enough time for the
culture to achieve nitrate-to-nitrite reduction. Further, the delicate
flavor profile of hams makes these products more susceptible to
flavor contributed by vegetable products.

The authors also pointed out that the concentration of celery
juice powder used (0.2, 0.35 and 0.4% on a total formulation
weight basis) could provide, with 100% nitrate-to-nitrite conver-
sion, maximum ingoing nitrite concentrations of 69, 120 and 139
ppm (meat block basis), respectively, based on the initial nitrate
concentration of 27,462 ppm in the celery powder. Because these
nitrite concentrations are, at best, significantly less than the 156–
200 ppm normally included in cured comminuted products or
injected products, it seems likely that product quality differences
could occur in some circumstances. It is also worth noting that the
USDA requires a minimum of 120 ppm ingoing nitrite for in-
jected bacon and cured meats labeled “Keep refrigerated” to assure
safety. Consequently, the actual amounts of nitrite formed from
nitrate when natural nitrate sources are used could be a concern
relative to microbiological safety. The shelf life of processed meats
manufactured with natural nitrate sources is generally shorter than
that of nitrite-cured products because less nitrite is present and
other typical preservatives such as phosphates, lactate, curing ac-
celerators and antioxidants are not included (Bacus, 2006).

Ingredients that might be considered as curing adjuncts for na-
tural or organic processed meats include vinegar, lemon juice solids,
and cherry powder. Acidulants such as vinegar have the potential
to accelerate nitrate reactions because of the impact of pH. Howev-
er, reducing pH in these products is also a concern for reduced
moisture retention because phosphates and many of the traditional
water binders cannot be used for natural or organic products.
Lemon juice solids or powder are typically significant sources of
citric acid which could have similar pH effects as vinegar. Cherry
powder, on the other hand, is high in ascorbic acid, which func-
tions as a strong nitrite reductant but does not have as great an
impact on pH.

An evaluation of a cured, Canadian-style bacon pork product
manufactured with a natural nitrate source (celery powder) and
with or without 0.28% cherry powder showed that including the
cherry powder reduced residual nitrite by about 50% (Baseler,
2007). Residual nitrite declined from 61 to 32 ppm during 12
weeks of storage for a nitrite-cured control, 18 to 10 ppm for the
celery powder treatment and 10 to 3 ppm for the celery pow-
der/cherry powder treatment. Addition of cherry powder did not
alter the product’s pH. Other product properties (color, lipid oxi-
dation) were not consistently different although the nitrite-cured
treatment showed greater redness (Hunter $a^*$ values) after about 4
weeks of storage.

Natural antioxidants such as rosemary may be used to provide
flavor protection and to retard lipid oxidation in processed meats.
However, these compounds do not contribute directly to ni-
trate/nitrite reactions in meat systems. Further, the nitrite gener-
ated from natural nitrate sources reported by Sindelar et al., (2007a,
2007b) was sufficient to provide strong antioxidant effects, similar
to those typically observed in nitrite-cured meats. Past research has
shown that as little as 50 ppm added nitrite has a highly significant
effect on lipid oxidation (Morrissey and Tichivangana, 1985). Thus,
relatively small amounts of nitrite formed from nitrate prob-
ably provide an important antioxidant role in natural and organic
processed meats.
Processes for Naturally Cured Meats

Most processors that use “natural curing” are following processing procedures that are generally similar to those processes that include chemical nitrites and nitrates. Naturally-cured products typically use natural sources for nitrate, but some natural ingredients may also contain nitrites. If sufficient nitrite is consistently available from a natural source, no changes in the normal process are required.

Naturally-cured meat products that use natural ingredients as a nitrate source need an ingredient that contains a relatively high natural nitrate content. When using a natural nitrate source, conversion of the nitrate to nitrite is required and this conversion is accomplished by specific microorganisms (with a nitrate reductase enzyme), as described earlier, that are also acceptable food ingredients. When using these microorganisms, the conversion process requires some time, with the specific amount of time depending upon the temperature, the environment, and the concentration of the reactants, namely the microorganisms and the naturally occurring nitrate. The conversion time can be decreased by increasing the reactant concentrations, with the amount of starter culture being the most critical variable.

In all natural curing processes, good distribution of both the nitrate source and the starter culture is essential to achieve uniform curing. The natural nitrate source, if dry, is usually either blended with the dry seasoning component for comminuted products, or added directly to curing brines. The starter culture commonly is diluted first with good quality water (i.e., distilled, or water low in chlorine or other bacteriocidal chemicals) prior to the addition to comminuted products (the USDA permits a maximum 0.5% combined water and starter culture without labeling the added water) or the starter culture may also be added directly to curing brines. Also, it is recommended that the starter culture should not be pre-blended with anything that might affect its viability (i.e., spices, salt), and hence its nitrate reducing activity. The naturally occurring nitrate is soluble, but because the starter culture is not soluble, being water dispersible, some agitation is recommended for brines to achieve optimal distribution in the meat product.

With curing brines, the pH of the brine is critical to achieving optimal natural curing as well as final product texture, because the phosphates or other buffering agents typically used with nitrite-added products cannot be included for products labeled natural or organic. Generally, low pH brines (i.e., <5.5) are not desirable, therefore the pH effect of any added natural ingredients should be considered. With comminuted meat products, the pH effect of directly-added ingredients is not as critical due to the buffering capacity of the meat.

Liquid sources of naturally occurring nitrates (vegetable juices) also are used but these ingredients pose some manufacturing issues. Typically, most of these liquids are not shelf stable, and are supplied in frozen form. Second, the added water that is a component of the juices must be considered.

Natural Cooked Sausage Products

A typical natural cooked sausage product formulation and process is shown below.

<table>
<thead>
<tr>
<th>Natural Hot Dog Formulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pork 72’s 52.60%</td>
</tr>
<tr>
<td>Beef 50’s 22.70</td>
</tr>
<tr>
<td>Water 20.30</td>
</tr>
<tr>
<td>Sea salt 1.28</td>
</tr>
<tr>
<td>Natural Hot Dog Seasoning 3.10</td>
</tr>
<tr>
<td>Cane sugar, natural flavors, sea salt, celery powder, onion powder, garlic powder, oleoresin paprika</td>
</tr>
<tr>
<td>Starter culture 0.02</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Natural Hot Dog Process</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) Grind meats through a 3/16-inch (4.8 mm) plate.</td>
</tr>
<tr>
<td>2) Mix starter culture with water totaling up to 0.50% of the total batch.</td>
</tr>
<tr>
<td>3) Mix/chop lean meats, adding in order, salt, ½ of the water, fatty meats, seasoning, and remaining water.</td>
</tr>
<tr>
<td>4) Add diluted starter culture.</td>
</tr>
<tr>
<td>5) Continue mixing/chopping until the meat blend temperature reaches 50–54°F (10–12°C).</td>
</tr>
<tr>
<td>6) Emulsify to 62–64°F (17–18°C).</td>
</tr>
<tr>
<td>7) Stuff and link.</td>
</tr>
<tr>
<td>8) Place on smokehouse rack and process using the smokehouse schedule.</td>
</tr>
<tr>
<td>a) 110°F (42°C) 60 minutes</td>
</tr>
<tr>
<td>b) 140°F (60°C) 20 minutes</td>
</tr>
<tr>
<td>c) 155°F (68°C) 30 minutes</td>
</tr>
<tr>
<td>d) 175°F (79°C) 30 minutes</td>
</tr>
<tr>
<td>e) 185°F/30% RH to 165°F (73°C) internal temperature</td>
</tr>
<tr>
<td>f) Shower</td>
</tr>
</tbody>
</table>

The formulation and process are essentially the same as a typical, nitrite-added product, except that celery powder is added as a natural nitrate source to replace the typical nitrite cure, the starter culture is added, and the smokehouse process allows for an “incubation” period of 1 hour at 110°F (42°C) to achieve the nitrate conversion to nitrite prior to a typical cook cycle.

Natural Hams

For injected products, such as natural hams, a typical formulation and process is shown below.

<table>
<thead>
<tr>
<th>Natural Ham Formulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water 79.135%</td>
</tr>
<tr>
<td>Natural Ham Seasoning 20.820</td>
</tr>
<tr>
<td>Sea salt, cane sugar, celery powder</td>
</tr>
<tr>
<td>Starter culture 0.045</td>
</tr>
</tbody>
</table>

The formulation and process are essentially the same as a typical, nitrite-added product, except that celery powder is added as a natural nitrate source to replace the typical nitrite cure, the starter culture is added, and the smokehouse process allows for an “incubation” period of 1 hour at 110°F (42°C) to achieve the nitrate conversion to nitrite prior to a typical cook cycle.
Natural Ham Process
1) Bone and trim pork, inside and outside pork rounds.
2) Dissolve and mix natural ham seasoning and starter culture into water prior to use.
3) Inject meat to 132% of green weight with the prepared pickle.
4) Macerate injected muscles on each side.
5) Tumble/massage under vacuum for a total of 5 hours. Tumble with 1/3 interval active and 2/3 intervals inactive (10 minutes on and 20 minutes off).
6) Stuff hams into pre-smoked netted casings.
7) Place hams in vacuum packager and evacuate (without packaging materials) to remove air.
8) Place hams on cook rack.
9) Smoke hams to internal temperature of 158°F (70°C) using the following smokehouse process.
10) Chill in cooler overnight (8–10 hours).
11) Remove netting before vacuum packaging.

Natural Ham Smokehouse Schedule
<table>
<thead>
<tr>
<th>Dry Bulb (°F)</th>
<th>Wet Bulb (°F)</th>
<th>RH (%)</th>
<th>Time (minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>165 (74°C)</td>
<td>115 (46°C)</td>
<td>22</td>
<td>45</td>
</tr>
<tr>
<td>165 (74°C)</td>
<td>115 (46°C)</td>
<td>22</td>
<td>15</td>
</tr>
<tr>
<td>165 (74°C)</td>
<td>115 (46°C)</td>
<td>22</td>
<td>30</td>
</tr>
<tr>
<td>165 (74°C)</td>
<td>115 (46°C)</td>
<td>22</td>
<td>60</td>
</tr>
<tr>
<td>175 (79°C)</td>
<td>155 (68°C)</td>
<td>59</td>
<td>30</td>
</tr>
<tr>
<td>180 (82°C)</td>
<td>180 (82°C)</td>
<td>100</td>
<td>55 Core temperature 158°F (70°C). Estimated 2 hours</td>
</tr>
</tbody>
</table>

For injected products, since the starter culture is not soluble, the physical injection process is critical for optimum distribution. The culture does not migrate in the meat, and thus poor distribution can result in uncured spots if the culture is not present. Generally, a relatively high injection percentage of brine is preferred.

In the above process, no “incubation” is required for the nitrate conversion due to the larger diameter of the ham pieces and more gradual “come up time” for the internal temperature. With smaller diameter products and rapid heat penetration, the heating process may have to be adjusted to achieve optimal nitrate conversion.

Natural Bacon Process
1) Trim pork bellies.
2) Prepare pickle prior to use.
3) Dissolve the following in water: sea salt, cane sugar, celery powder, and starter culture.
4) Pump pork bellies to 115% of green weight.
5) Place the pumped pork bellies on bacon hooks and smokehouse process.
6) Chill and slice.

Natural Bacon Smokehouse Schedule
<table>
<thead>
<tr>
<th>Dry Bulb (°F)</th>
<th>Wet Bulb (°F)</th>
<th>RH (%)</th>
<th>Time (minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>110 (42°C)</td>
<td>92 (35°C)</td>
<td>70</td>
<td>75</td>
</tr>
<tr>
<td>145 (63°C)</td>
<td>-</td>
<td>-</td>
<td>60</td>
</tr>
<tr>
<td>145 (63°C)</td>
<td>-</td>
<td>-</td>
<td>15</td>
</tr>
<tr>
<td>134 (57°C)</td>
<td>-</td>
<td>-</td>
<td>90</td>
</tr>
<tr>
<td>140 (60°C)</td>
<td>120 (49°C)</td>
<td>55</td>
<td>Core temperature 128°F (53°C). Estimated 180 minutes</td>
</tr>
</tbody>
</table>

Generally, since injected pork bellies are relatively thin in diameter, a short “incubation” period at 110–115°F (42–46°C) is recommended prior to the common heating cycle. Some processors have found with natural bacon that their normal bacon process provides adequate incubation time during the “come up” heating phase. Because bacon is not fully cooked, relatively high bacterial counts from the added starter culture will remain in the product.

Natural Pepperoni
Natural fermented sausages do not require any adjustments in processing since an “incubation” step (fermentation phase) is already incorporated into their normal process to allow the added starter culture to reduce the sugars to lactic acid and other metabolites. Since most meat starter cultures, particularly in the United States, consist of lactic acid producing bacteria only, it is imperative to confirm that the added starter culture contains a mixed culture with one culture to ferment the added sugars and at least one other culture to reduce the naturally occurring nitrates to nitrite. Many mixed starter cultures are available to accomplish both functions, or the nitrate-reducing culture can be added “on top” of the existing acid-producing culture.

Natural Pepperoni Formulation
<table>
<thead>
<tr>
<th>Component</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pork 72</td>
<td>66.08%</td>
</tr>
<tr>
<td>Beef 50</td>
<td>12.40%</td>
</tr>
<tr>
<td>Beef 65</td>
<td>16.20%</td>
</tr>
<tr>
<td>Natural Pepperoni Seasoning</td>
<td>5.00</td>
</tr>
<tr>
<td>Sea Salt, Natural Cane Sugar, Spices, Natural Flavorings (including celery powder), Garlic Powder, Paprika Extractives</td>
<td></td>
</tr>
<tr>
<td>Meat starter culture (lactic acid production)</td>
<td>0.30</td>
</tr>
<tr>
<td>Meat starter culture (nitrate conversion)</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Natural Bacon Formulation
<table>
<thead>
<tr>
<th>Component</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>66.38%</td>
</tr>
<tr>
<td>Sea salt</td>
<td>22.00%</td>
</tr>
<tr>
<td>Cane sugar</td>
<td>10.40%</td>
</tr>
<tr>
<td>Celery powder</td>
<td>1.20%</td>
</tr>
<tr>
<td>Starter culture</td>
<td>0.02</td>
</tr>
</tbody>
</table>

American Meat Science Association
Natural Pepperoni Process

1) Temper fresh/frozen meats to 26–28°F (−3 to −2°C). Grind or chop to 15–20 mm.
2) Mix meats in paddle-type mixer OR chop in silent cutter with mixing speed, adding in sequence, seasoning and starter cultures (pre-diluted in water to 0.5% batch weight), with minimum mixing/cutting time to achieve good distribution. 
3) Regrind through a 3–4 mm plate OR chop to similar particle size.
4) Stuff into regular, flat stock fibrous casings (50–53 mm for slicing) or collagen casings so as to minimize “smear” (meat temperature @ 26–28°F (−3 to −2°C)).
5) Equilibration for a minimum of 2 hours @ 71–72°F (22–23°C).
6) Fermentation:
   a) 95–104°F (35–40°C), 85% RH to pH < 5.0 (8–10 hours)
   b) 120–122°F (49–50°C), 60% RH (1 hour)
   c) 129–131°F (54–55°C), 60% RH to 128°F (53°C) internal temperature, hold 1 hour
7) Hot shower and cool to ambient temperature.
8) Dry room @ 52–55°F (11–13°C), 65–72% RH to moisture/protein ratio of 1.6/1.0 (10–14 days, 68–72% yield).

Current Issues with Natural and Organic Cured Meats

Regulatory

The current regulatory issues concerning “organic” meat products are well defined by the USDA Agricultural Marketing Service, thus processors desiring to make such products must adhere to a fixed set of regulations outlining permitted ingredients. With “natural” meat products, however, the rules for permitted ingredients have recently become more confusing. Until August, 2005, “natural” simply meant “minimally processed” and “no artificial ingredients”, with any meat source considered natural. The “natural” rules were based on USDA Policy Memo 055, prepared in 1982, and focused on the process and the non-meat ingredients. With revisions outlined in the USDA Food Standards and Labeling Policy Book (USDA 2005), additional ingredients, including sodium lactate (from a corn source), cane sugar, and natural flavorings from oleoresins and extractives were permitted to be labeled as “natural”. Additionally, the Policy Book referenced 7 CFR NOP Final Report, Part 205.601 through 205.606 for acceptable ingredients allowed for all natural claims.

A petition submitted to the USDA in October, 2006, suggested that the 2005 revisions to the agency’s “natural” policy created inconsistencies by allowing foods carrying the “natural” label to contain synthetic ingredients and preservatives, deceiving consumers and eroding the “natural” label to a meaningless marketing policy. Much of the concern expressed by the petitioner was the allowance of sodium and potassium lactates in “natural” products, since these ingredients would be considered “chemical preservatives”. Also, by allowing any ingredients on the OFPA List (for “organic” products), even some synthetic ingredients on the list were permitted in “natural” products. The petition to the USDA proposed that extensive rulemaking should be initiated for meat and poultry products labeled as “natural” in much the same way as had been done with products labeled as “organic”.

Consequently, USDA reversed its position on the use of lactates, categorizing these ingredients as “chemical preservatives”, and notified processors using them that they must remove lactates from their products within 30 days, unless the processor could demonstrate that such use was not as a preservative. Additionally, the USDA clarified its reference to the OFPA List, only permitting those ingredients on the List that would be considered “natural”. The rule making for “natural” has begun, with a public meeting in Washington in December, 2006, and a comment period extended to March 5, 2007.

The issue of lactates as “chemical preservatives” also raised the issue of dual-function ingredients, whereby the ingredient may be considered as a natural ingredient for flavor and/or function, but can also have a dual function as a “natural” preservative. The issue of “natural preservative” vs. “chemical preservative” has not been defined, as yet. By strict interpretation, any “preservative” used in a HACCP Program, that allows a processor to classify their product in Alternative 1 or Alternative 2, in regard to Listeria monocytogenes control, would not be permitted to be labeled as “natural”.

Many natural compounds that exist in the environment can serve to inhibit microorganisms, retard oxidation, and thus “preserve” the product and would be valuable ingredients in food products that are labeled as “natural”. Until this issue of “natural” vs. “chemical” preservatives is resolved, the current regulatory environment is retarding innovative product development and may compromise food safety as well.

Manufacturing

When manufacturing “natural” and “organic” meat products using natural ingredients, the inherent variability of natural ingredients must be considered. In the natural curing process, whereby naturally-occurring nitrates are converted by starter cultures to nitrites, the concentration of the nitrate in the source will affect the degree of curing as well as the amount of nitrate-reducing activity of the starter culture. Typically, in products cured with direct addition of sodium nitrite, the ingoing nitrite is regulated at 156 ppm in most meat products and at 120 ppm for bacon. In naturally-cured meat products, the ingoing nitrate level is most often between 40–60 ppm, thus there is, at best, significantly less nitrite in these products than in the typical nitrite-cured products. Measurable residual nitrite in both types of cured products are often similar with no noticeable differences in color and stability. However, this will be very dependent upon actual nitrite formation in the product during the nitrate reduction phase of the process. While reduced shelf-life has been observed in naturally cured meats, a significant part of the change is probably due to the lack of other traditional ingredients that are not permitted in “natural”
products (i.e. phosphate, ascorbate, erythorbate, citric acid, and synthetic antioxidants).

Marketing

The issue of the consumer's understanding of what is meant by "natural” meat products is difficult to define. Many consumers may not comprehend that natural ingredients often contain naturally occurring chemicals virtually identical in chemical nature to those chemicals synthetically produced.

With regard to natural curing, many foods, particularly vegetables, contain naturally occurring nitrates. It is generally accepted that less than 5% of human consumption of nitrate and nitrite is due to meat products. The majority of nitrates consumed are from vegetable products (Archer, 2002). The current USDA concern with “naturally cured” meat and poultry products is that these products often contain residual nitrates and nitrites, even though correctly labeled as “no nitrates or nitrites added”. According to the Code of Federal Regulations 9 CFR 319.2, the processor has no choice but to label such products (i.e., "… to which nitrite or nitrate is permitted or required to be added...") as “uncured” and no “nitrates or nitrites added”, even though the processor may be using a natural curing process. The USDA attempted to remedy this issue by requiring a disclaimer (no nitrates or nitrites added … except those naturally present in ______) on such products that contain ingredients that may contain naturally occurring nitrates and nitrites, but this adds to consumer confusion and labeling inconsistencies. The individual USDA label reviewer must determine in each case if the added ingredients in the meat product label submittal could contain nitrates and/or nitrites, which is possible for a multitude of ingredients. Vegetables, bouillons, and fruits are ingredients that have been used in combination with meat and poultry products for centuries and have probably contributed nitrate/nitrite to these products for as long as they have been used. Also, it is possible for environmental nitrates and nitrites to contaminate the product during processing (i.e., water source, smoking).

To provide the consumer with the most accurate information, more appropriate labeling would be to use a term such as “naturally cured” because the products are truly cured with naturally occurring nitrate and/or nitrite added in the form of natural ingredients, and eliminate the “uncured” and “no nitrates or nitrites added” requirement. This would require modifying the federal regulations in 9 CFR 319.2. Residual nitrite is the real issue, not the cured meat pigments that result from the various nitrite reactions. Replacing the disclaimer with the footnote “naturally occurring nitrates may be present”, would adequately inform the consumers of the potential existence of nitrite in the "naturally cured" products. Another alternative would be to allow processors to avoid the disclaimer or footnote if they can prove that they can control their process to eliminate any residual nitrates, which is the primary concern for many consumers, particularly in bacon.

Quality

The quality characteristics expected of traditional cured meats that are unique to these products include the reddish-pink color of cooked denatured nitrosylhemochrome, a flavor that is distinct from uncured products, and long-term flavor protection resulting from the strong antioxidant effect of nitrite on meat systems. The fixation of desirable color is the first and most obvious effect of nitrite when added to meat and is considered an essential function because color is a critical component affecting consumer retail purchases (Cornforth and Jayasingh, 2004). As little as 2–14 ppm of nitrite (depending on species) can induce pink coloration in cooked meats though at these levels the color is often sporadic and likely to fade with time. Extensive research in the 1970’s showed that 25–50 ppm of ingoing nitrite was adequate to develop relatively stable cured color (National Academy of Sciences, 1982). While there are indications that cured color may be less intense with 40–50 ppm of nitrite instead of 150–200 ppm depending on product type, 40–50 ppm is generally considered adequate for cured color development in most products. Thus, it would appear that cured color development can be achieved relatively easily in processed meat using natural sources of nitrate and a nitrate-reducing culture. A related question, however, concerns the long term stability of the cured color formed in these products. One of the difficulties with assessing potential cured color intensity or stability with nitrate-based cures is that the absolute amount of nitrite formed from nitrate cannot be determined due to the reactive nature of nitrite in meat, and can only be estimated from the amount of nitrate that is depleted. Sindelar et al. (2007a), for example, reported that small diameter sausage made with celery powder and culture had 9.3–31.9 ppm of residual nitrate remaining when 69 ppm of nitrate was added as part of the celery powder, and 12.2–81.4 ppm remaining when 139 ppm was added. So, if 100% of the nitrate that was depleted was, in fact, reduced to nitrite, the ingoing nitrite concentrations ranged from 37 to 127 ppm. This is sufficient nitrite to generate desirable cured meat color characteristics in most processed meat products. Similar results were observed with hams (Sindelar, 2007b) where the residual nitrate concentrations suggested formation of nitrite in the range of 45 to 119 ppm. Thus, the quality of cured color in terms of intensity and stability is not likely to be a major issue in processed meats using natural sources of nitrate if appropriate processing procedures are followed to achieve nitrate reduction, and if adequate packaging (oxygen removal by vacuum or gas flushing and high oxygen-barrier films) is used (Møller et al., 2003).

Cured flavor is an important quality attribute of cured meats that is derived from the addition of nitrite, although the chemical nature of the flavor has never been established. It is clear, however, that relatively low concentrations of nitrite result in significant cured flavor. Several researchers have reported acceptable cured meat flavor in products formulated with 40 ppm of ingoing nitrite (Pegg and Shahidi, 2000). In a series of reports, MacDonald et al., (1980a, 1980b, 1980c) concluded that addition of 50 ppm of nitrite to hams was sufficient to produce significant cured meat flavor and antioxidant protection. Thus, in addition to color,
appears that 40–50 ppm or more of ingoing nitrite will result in a significant flavor contribution to cured meat.

The third quality contribution of nitrite to cured meat is the often-overlooked, but highly effective role of nitrite as an antioxidant. Whether nitrite, nitric oxide or some reaction products of these compounds are responsible for the antioxidant function is not clear despite extensive past research. It is clear, however, that nitrite is again effective at relatively low concentrations. Morrissey and Techivangana (1985), for example, using cooked, ground beef, pork, chicken and fish muscle, reported that 50 ppm of nitrite reduced TBA values by 50–64% for beef, pork and chicken, and about 35% for fish. Nitrite concentrations of 100 ppm resulted in TBA reductions of 57–72%, and 200 ppm reduced TBA values by 87–91%. There was a very clear relationship between saturated:unsaturated fat ratios and the TBA values, with more unsaturated fats resulting in greater TBA values regardless of the nitrite concentration. While nitrite is effective as an antioxidant at 50 ppm, it is more effective at greater concentrations up to 200 ppm. The nitrite concentration becomes increasingly important for meat products with greater amounts of unsaturated lipids. Further, the antioxidant function of nitrite in cured meat, while highly effective, is not as unique as the color and flavor contributions. There are a number of antioxidants including natural compounds that can protect meat lipids from oxidation and flavor deterioration.

If at least 50 ppm of nitrite is formed from nitrate during processing of meat products with natural nitrate sources, it appears that the typical quality characteristics expected of cured meat (color, flavor, flavor stability) will be achieved. A question that is more difficult to answer is the long-term stability of those quality characteristics. It is well recognized that when nitrite is fully depleted from cured meat, color fading and flavor changes typically occur. Some residual nitrite is essential to maintaining typical cured meat properties during extended product storage. It appears that the 5–15 ppm residual nitrite observed in commercial cured meats (Cassens, 1997b), is a reasonable indicator for long-term stability. It is important to keep in mind that packaging and environmental conditions, particularly temperature and exposure to light, are critical to long-term cured meat quality, and become more critical when residual nitrite is reduced.

Safety

The safety of processed meats that simulate traditional cured meats by using natural sources of nitrate is a significant issue for two reasons; first, nitrite is a very effective antimicrobial agent, particularly for preventing toxin production by Clostridium botulinum and second; residual nitrite concentration is a well-known risk factor in the potential formation of carcinogenic nitrosamines. In both cases, ingoing and residual nitrite concentrations must be carefully controlled to provide product safety.

The antimicrobial role of nitrite in cured meat has been well documented. Christiansen (1980), in a review of botulinal inhibition by nitrite, concluded "that any change in nitrite usage which reduces the level of residual nitrite or increases the rate of nitrite depletion could increase the above mentioned (botulinal) theoretical risk.” The issue for processed meats that use natural sources of nitrate is that the true amount of nitrite formed is unknown and impossible to measure because nitrite reacts quickly with meat components. While the amount of detectable residual nitrite in these products is significant, it is often less than that found in nitrite-cured products (Sindelar et al., 2007a, 2007b) depending on processing conditions. On the other hand, the nitrite reactions means that there are variable pools of nitrite-modified compounds in cured meat that remain available as reactive sources of nitric oxide (Kanner and Juven, 1980; Möller and Skibsted, 2002). Consequently, the microbial safety of processed meats manufactured with natural sources of nitrate is very difficult to assess without microbiological challenge studies. This is a very significant current research need that remains open in assessing the safety of these products.

The second potential safety issue that should be considered with these products is the implications of higher-than-usual nitrite concentrations. Elevated residual nitrite in bacon is a potential risk for nitrosamine formation and actual ingoing nitrite concentrations need to be carefully controlled to avoid this potential problem. The nature of the time-temperature relationship for reduction of nitrate to nitrite by a starter culture makes the concentration of nitrite a variable entity. Further, vegetable products are recognized as extremely variable in nitrate content as a result of different environmental conditions that occur during plant growth (National Academy of Sciences, 1981). Consequently, more information is needed on the best means by which to control nitrite formation in processed meats manufactured with natural sources of nitrate to assure that excess concentrations of nitrite do not become a safety issue.

References


Baseler, L.J. 2006. Unpublished data. Iowa State University, Ames, IA.


Sebranek, J.G. 2006. Unpublished data. Iowa State University, Ames, IA.


Sindelar, J.J. 2006b. Unpublished data. Iowa State University, Ames, IA.


The American Meat Science Association is a broad-reaching organization of individuals that discovers, develops, and disseminates its collective meat science knowledge to provide leadership, education, and professional development. AMSA is a nonprofit organization composed of over 800 scientists and students representing academia, industry, government and allied organizations. AMSA is governed by a member-elected Board of Directors. AMSA was established in 1965 as a result of a series of meetings called the Reciprocal Meat Conferences organized by the National Live Stock and Meat Board.
Joint AMI-FMI Meeting
Preparations
An Industry Survey
For the Presence of \textit{Listeria monocytogenes}
In Grocery Deli Operations

A Pre-Proposal Prepared for
The American Meat Institute
Foundation

Submitted by
Ann Marie McNamara, Sc.D.
Vice President, Food Safety and
Scientific Affairs
Silliker, Inc.

March 25, 2005
Objective

USDA’s Food Safety and Inspection Service (FSIS) has determined that their jurisdiction under the Meat and Poultry Inspection Acts extends through distribution and retail. USDA is currently funding a NIFSI study in which 8,000 deli and manufacturer packaged, RTE meat and poultry products from grocery stores will be tested for the presence of *Listeria monocytogenes* (Lm). This study is being led by Dr. Ann Draughon, University of Tennessee, and will be completed in October 2005.

The design of this NIFSI study allows only for the sampling of meat and poultry products sliced at deli counters; collection of whole, intact chubs and collection of pegboard meats, presliced and packaged by the manufacturer. Since the investigators are visiting these stores unannounced and surreptitiously, they are unable to take concomitant environmental samples, independently verify temperature readings, or question store personnel on product handling practices. These omissions are serious flaws in this study since any Lm positive findings cannot be traced back to a root cause. At best, this study will be able to conclude that a given number of samples out of 8,000 samples were positive. These results may only incite concern by the general public and provide no answers to retail operators for the cause of the contamination or possible mitigation measures.

The limitations of this study and the potential for sensationalism of the results without linkages to causation, was discussed at the March 2-3, 2005 AMI Scientific Affairs Advisory Committee Meeting, Atlanta, GA. Committee members believed that a properly designed study would address the question of the impact of deli operations on product contamination. SAC members identified the following data gaps in the current NIFSI study that will be addressed by this new survey:

- Environmental deli samples should be considered.
- The exterior of intact packages should be sampled.
- *Listeria* inhibitors in the product sliced at deli should be known if possible.
- Retail cooperators are needed since deli access will be required to conduct the study appropriately.
- Use Cook-in-Bag product. Consider obtaining a list of products (brands) that are known to have been cooked in bag. This could determine if inhibitors were present in the products and determine if Lm transfer is occurring at the retail slicer.
- Design of study should be geared to assess *Listeria* transfer.
- General deli housekeeping and SOPs within the deli should be recorded.

This study will survey 100 grocery deli operations throughout the U.S. The design will incorporate the collection of environmental samples, cook-in-bag product sliced at the deli, verification of temperature recording devices, and a survey of deli housekeeping and SOPs. The intent is to determine if Lm is present in delis and to assess the possibility of *Listeria* transfer through the slicing of cook-in-bag meats. Verification of temperature recordings and surveys of retail food handling practices may provide insight into any contamination found and may provide possible mitigation factors lacking in the NIFSI study. This study will be blinded on multiple levels so that the identity of groceries, manufacturers of cook-in-bag products to be sliced, and
identity of surveyors will not be known to the laboratories processing the samples and to the principal investigator analyzing the data. Data will be blinded in a way to make traceback impossible. This study will take 4 months to complete, and results will be available prior to the completion of the NIFSI study.

This study will require the cooperation of AMI processed meat and poultry manufacturers and grocery operators to be successful. Participating manufacturers will need to provide the names of cook-in-bag products found in a typical deli and the additives/post-packaging interventions used to make these safe products. All cook-in-bag products will be considered to be free of *Listeria* from the manufacturer and sliced at deli to show the potential for cross-contamination at the deli and transfer of *Listeria* through slicing. Grocery operators will need to provide a letter of introduction for Silliker sample collectors, complete with contact information of a designated food safety/regulatory person at the grocery chain, to assure cooperation during unannounced visits.

General descriptions for the contents of the three sections of this study are detailed here. If AMI Foundation Members accept this pre-proposal, a full proposal and survey instrument will be developed.

**Note:** This pre-proposal tests the deli environment and sliced product for Lm. This same study can be designed to detect *Listeria* species if there is a concern about regulatory issues. All samples will be blinded so no trace back will be possible. The NFPA retail survey and now the NIFSI study are being conducted without concommitant regulatory action on Lm positive products.

**A. Collection of Deli-Sliced Product Samples**

Upon each site visit, the sample collector will request that 0.5 lb (minimum of 15 slices) of a cook-in-bag product identified by participating manufacturers will be sliced in their presence at the deli. The purpose will be to assess potential Lm transfer. Samples will be shipped refrigerated overnight to a participating Silliker laboratory. Processing of samples will follow the NFPA protocol used in their retail survey. In brief, 25g of product will be enriched in UVM broth for 24h, transferred to MOPS-BLEB for 24h, and analyzed by DuPont Qualicon’s ABAX PCR test for Lm. The remaining portion will be held for quantitation by MPN analysis and biochemical confirmation of isolates (D.E. Gombas et al\(^1\)). One product will be sliced at each visit (100 products, 100 visits total).

**B. Collection of Environmental Samples and Verification of Temperature Recordings**

Environmental sponges of the slicer, food contact surfaces, and the deli case will be collected for the presence of Lm. Sponges will be collected in DE Neutralizing broth and shipped to participating Silliker laboratories for processing. Sample collection kits will be prepared by the Silliker Corporate Research Laboratory and sent to Silliker sample collectors.

Tentatively identified environmental sample sites include the following. Further identification/modification environmental sites by the AMI Foundation members are welcomed.

1. Slicer-blade, back plate, meat collection areas, guard, handle.
2. Food contact surfaces – weighing station, cutting boars/preparation tables, trays for transporting products.

3. Deli Case – trays or flooring of case, door handles, door track, drains beneath/around deli case.


Environmental samples will be processed using current Qualicon ABAX recommendations: UVM for 24h incubation then MOPS-BLEB for 24h incubation. (Note: Neither the NFPA nor the NIFSI study collected environmental samples.) Positive samples will be quantitated and confirmed biochemically.

Verification of temperature readings by temperature recording devices in the deli area will be performed by the sample collector using a calibrated thermometer. Readings will be taken of the deli case and product storage refrigerators.

C. Deli Practices and SOP Survey.

If this pre-proposal is accepted by the AMI Foundation members, a complete survey instrument will be developed. This survey will be conducted by the sample collector at the time of the grocery store visit. Deli personnel will be interviewed on deli practices and not general store managers. This survey is intended to determine current deli food handling practices and SOPs that may correlate to any positive Listeria environmental or product samples. This survey will include questions on products offered at deli counters (deli salads, blue-veined or soft-mold-ripened cheeses, cooked products such as rotisserie chickens) separation of raw and RTE products and preparation areas, shelf-life dating of products, age and manufacturers of slicers, certification of refrigeration thermometers, cleaners and sanitizers used, sanitation schedule for food utensils and food contact surfaces, and safe food handling practices.

Silliker is uniquely positioned to conduct such a study quickly and competently. We have sixteen consultants and technical sales managers trained in the collection of environmental and product samples and their shipping requirements. These Silliker personnel are located in Illinois, Wisconsin, Iowa, Minnesota, Pennsylvania, New Jersey, New York, Georgia, Texas, and Northern and Southern California. They can be mobilized to collect samples in adjoining states. Silliker has conducted large marketbasket surveys for trade organizations, such as AMI and NCBA, and individual companies.

Fees

Cost of swab kits, collection of 15 environmental and 1 product samples, shipment of samples to lab, processing of samples by PCR, results generation, survey administration to deli personnel, and temperature verifications = $603.00 per site visit or $60,300.00 per 100 visits (number of visits may be modified by AMI Foundation members)
MPN analysis for hypothesized 25% positive samples = $6,250.00

Survey preparation, review by AMI Foundation, correlation meeting for sample collectors = $4,750.00

Data analysis and report = $6,000.00

Total for 100 site visits = $77,300.00

References:

1. Elliot Ryser

Appendix A. Blinding Scheme for AMI-FMI Retail Listeria Study Approved by AMI and FMI

Goal: To protect the identity of the retail stores participating in the retail Listeria study.

Protocol: Sample collectors will be assigned sufficient numbers to represent the number of stores being visited. Each sample collector will randomly assign a number to a retail store at the time of the visit. That number will be used on each sample collection form, retail survey, and sample labels to protect the identity of the retail store. This number will follow the materials through the laboratory to the reports to the statistician. The statistician will assign a new number to the data collected from each store at the time that the data is being entered into the database. Original data containing the original, randomly assigned number is then destroyed to prevent any traceback. All data containing the new number will be in numerical order based on the time of receipt of the data.

An example of this procedure is as follows: Sample collector A is assigned responsibility for sampling 10 retail stores. He will be assigned the numbers 40 to 50 to represent these stores. The sample collector will randomly assign one of these numbers to each retail store at the time of the visit. Only he will know the identity of the store. That number, for example number 43, will be used on each sample collection form, retail survey, and sample labels from that store. This number will follow the materials through the laboratory to the laboratory reports to the statistician. Neither the laboratory nor the statistician will know the identity of the store. The statistician will assign a new number to the data collected from each store at the time that the data is being entered into the database. Original data containing the original, randomly assigned number is then destroyed to prevent any traceback. For instance, if the data labeled 43 is the 67th data set to arrive at the statistician, it will now become 67, the data is entered into the database using this new number, and the original number and documents discarded. In this manner, the original store has been protected through two assignments of new identifiers (numbers) that do not link back in any way to the original store.
Appendix B. Sample sites in AMI-FMI Listeria in Retail Study vs. New York Listeria in Retail Study

<table>
<thead>
<tr>
<th>AMI – FMI Sample Sites</th>
<th>NY Sample Sites^a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slicer – blade</td>
<td>Slicer</td>
</tr>
<tr>
<td>Slicer – back plate</td>
<td>Sponge from deli case</td>
</tr>
<tr>
<td>Slicer – meat collection areas</td>
<td>Sponge from sink interior</td>
</tr>
<tr>
<td>Slicer – guard</td>
<td>Drain in deli area</td>
</tr>
<tr>
<td>Slicer – handle</td>
<td>Drain in raw meat area</td>
</tr>
<tr>
<td>Weighing station</td>
<td>Drain in seafood area</td>
</tr>
<tr>
<td>Cutting boards/preparation tables</td>
<td>Floor in dry isle (canned goods)</td>
</tr>
<tr>
<td>Trays for transporting products</td>
<td>Sponge from dairy case</td>
</tr>
<tr>
<td>Deli case trays/flooring</td>
<td>Sponge of wheels from 4 grocery carts</td>
</tr>
<tr>
<td>Deli case door handles</td>
<td>Drain in produce preparation area</td>
</tr>
<tr>
<td>Deli case door track</td>
<td>Drain in restaurant/cafes</td>
</tr>
<tr>
<td>Drains beneath/around deli case</td>
<td></td>
</tr>
</tbody>
</table>

^a Sites cited are for stores where sliced deli meats are sampled; if store-made salads are produced, utensils used to prepare salads are sampled instead of the slicer. All other sites are the same.
Cross-Contamination between Processing Equipment and Deli Meats by *Listeria monocytogenes*

CHIA-MIN LIN,† KAZUE TAKEUCHI,† LEI ZHANG,‡ CYNTHIA B. DOHM,‡ JOSEPH D. MEYER,‡ PAUL A. HALL,‡ AND MICHAEL P. DOYLE†*  

†Center for Food Safety, University of Georgia, 1109 Experiment Street, Griffin, Georgia 30223-1797; and ‡Kraft Foods, 801 Waukegan Road, Glenview, Illinois 60025, USA  

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ABSTRACT

Contamination of luncheon meats by *Listeria monocytogenes* has resulted in outbreaks of listeriosis and major product recalls. Listeriae can survive on processing equipment such as meat slicers which serve as a potential contamination source. This study was conducted to determine (i) the dynamics of cross-contamination of *L. monocytogenes* from a commercial slicer and associated equipment onto sliced meat products, (ii) the influence of sample size on the efficacy of the BAX-PCR and U.S. Department of Agriculture–Food Safety and Inspection Service enrichment culture assays to detect *L. monocytogenes* on deli meat, and (iii) the fate of *L. monocytogenes* on sliced deli meats of different types during refrigerated storage. Three types of deli meats, uncured oven-roasted turkey, salami, and bologna containing sodium diacetate and potassium lactate, were tested. A five-strain mixture of *L. monocytogenes* was inoculated at ca.10⁰ CFU onto the blade of a commercial slicer. Five consecutive meat slices were packed per package, then vacuum sealed, stored at 4°C, and sampled at 1 and 30 days postslicing. Two sample sizes, 25 g and contents of the entire package of meat, were assayed. Total numbers of *L. monocytogenes*–positive samples, including the two sample sizes and two sampling times, were 80, 9, and 3 for turkey, salami, and bologna, respectively. A higher percentage of turkey meat samples were *L. monocytogenes* positive when contents of the entire package were assayed than when the 25-g sample was assayed (12.5 and 7.5%, respectively). Lower inoculum populations of ca. 10¹ or 10² CFU of *L. monocytogenes* on the slicer blade were used for an additional evaluation of oven-roasted turkey using two additional sampling times of 60 and 90 days postslicing. *L. monocytogenes*–positive samples were not detected until 60 days postslicing, and more positive samples were detected at 90 days than at 60 days postslicing. When BAX-PCR and enrichment culture assays were compared, 12, 8, and 2 *L. monocytogenes*–positive samples were detected by both the enrichment culture and BAX-PCR, BAX-PCR only, and enrichment culture only assays, respectively. The number of *L. monocytogenes*–positive samples and *L. monocytogenes* counts increased during storage of turkey meat but decreased for salami and bologna. Significantly more turkey samples were *L. monocytogenes* positive when the contents of the entire package were sampled than when 25 g was sampled. Our results indicate that *L. monocytogenes* can be transferred from a contaminated slicer onto meats and can survive or grow better on uncured oven-roasted turkey than on salami or bologna with preservatives. Higher *L. monocytogenes* cell numbers inoculated on the slicer blade resulted in more *L. monocytogenes*–positive sliced meat samples. In addition, the BAX-PCR assay was better than the enrichment culture assay at detecting *L. monocytogenes* on turkey meat (*P* < 0.05).

Although the prevalence of *Listeria monocytogenes* in ready-to-eat meat and poultry products has steadily decreased from 1990 to 2002 (25), two major multistate outbreaks resulting from *L. monocytogenes* contamination of deli meats occurred in 1998 and 2002 (6, 7). *L. monocytogenes* can grow at refrigeration temperature and is considered to be widely distributed because it can be isolated, although generally infrequently, from many locations in food processing plants (19). It had been suggested that contaminated raw materials introduce into processing plants *L. monocytogenes*, which is then transferred onto finished products. However, results of studies using molecular subtyping techniques to monitor the transmission of listeriae in food-processing facilities revealed that environmental contamination, not raw materials, is the most frequent source of *L. monocytogenes* in finished products (2, 4, 15, 16, 18). For example, in a study of *L. monocytogenes* isolates obtained in meat and sausage processing plants, two genetic clusters, A and B, were identified, of which almost all isolates from fresh meat belonged to cluster B and all isolates except one on finished products were in cluster A (16). Similarly, a 7-year study in an ice cream plant revealed that no *L. monocytogenes* was isolated from raw materials, but isolates with the same pulsed-field gel electrophoresis (PFGE) pattern were obtained from the production environment and finished products (15). A study of a rainbow trout processing plant revealed that predominant *L. monocytogenes* isolates from finished products were associated
with brining and slicing operations, not raw materials (2). Hence, the processing plant environment appears to be a greater source of *L. monocytogenes* contamination of finished product than of raw materials. In addition, *L. monocytogenes* can adhere to most materials that comprise food processing equipment (3, 13), including equipment used in deli meat plants. Slicing equipment, therefore, is a potential vehicle of cross-contamination by *L. monocytogenes* during meat processing. However, little information is available regarding this route of *Listeria* transmission.

There is considerable variability in growth potential of *L. monocytogenes* on different types of vacuum-packaged deli meats at refrigeration temperature (9). Additives such as sodium diacetate and potassium lactate currently are being added to certain deli meats to inhibit the growth of *L. monocytogenes* (20, 23). However, little is known about the fate of *L. monocytogenes* on different types of deli meat contaminated under processing conditions such as a contaminated meat slicer.

Sample size has been determined to be an influential factor in detecting *L. monocytogenes* in foods. A higher detection rate of *L. monocytogenes* on frankfurters was obtained by assaying the rinse of contents of the entire package rather than 25 g as a sample unit (12). In addition, molecular-based pathogen detection methods such as PCR have been developed to detect *L. monocytogenes* in foods. Comparative studies in which *L. monocytogenes* was inoculated directly onto food samples have been conducted to determine efficacy of enrichment culture and BAX-PCR assays (DuPont Qualicon, Wilmington, Del.) for detecting *L. monocytogenes* (10, 11, 21, 22). The objectives of this study were to determine (i) the dynamics of the transfer of *L. monocytogenes* from a contaminated commercial slicer and associated equipment onto different types of deli meats, (ii) the influence of sample size and the efficacy of BAX-PCR and the U.S. Department of Agriculture–Food Safety and Inspection Service (USDA-FSIS) enrichment culture assays to detect *L. monocytogenes* on deli meat, and (iii) the fate of *L. monocytogenes* on sliced deli meats of different types during refrigerated storage.

**MATERIALS AND METHODS**

**Bacterial culture.** A five-strain mixture of *L. monocytogenes* (H7550, an isolate from frankfurters; Coleslaw, an isolate from coleslaw; G3982, an isolate from Mexican-style cheese; OM, an isolate from a deli meat; and Plantation, an environmental isolate from a meat processing plant) was used. Strains H7550, G3982, and Coleslaw were from the culture collection of the Center for Food Safety, and Plantation and OM were provided by Kraft Foods. *L. monocytogenes* strains were maintained at −80°C, and each month a fresh culture was streaked onto tryptic soy agar with 0.6% yeast extract (TSAYE; Difco, Becton Dickinson, Sparks, Md.). Each strain was cultured separately in 10 ml of brain heart infusion broth (Difco, Becton Dickinson) at 37°C for three consecutive 24-h intervals before each trial. After incubation, each culture was sedimented by centrifugation at 4000 × g for 15 min, then washed twice with 10 ml of Butterfield’s phosphate buffer (pH 7.2) and resuspended in 8 ml of Butterfield’s phosphate buffer. An equal portion of each *L. monocytogenes* strain was then serially diluted (1:10) in 5% horse serum albumen (pH 7.3; Difco, Becton Dickinson). *L. monocytogenes* cell numbers in the inoculum were determined by plating dilutions onto TSAYE and incubating plates at 37°C for 24 h.

**Meat slicing and inoculation.** Three different types of deli meats, including uncured oven-roasted turkey, salami, and beef bologna, were used. The beef bologna contained 1.45% potassium lactate and 0.1% sodium diacetate, which are growth inhibitors of *L. monocytogenes*. The deli meat was held at −1°C before slicing. A meat loaf was peeled and loaded vertically onto a commercial-scale meat slicer (model 1085SS, Great Lakes Corporation, Chicago, Ill.). The meat loaf was sliced by a horizontally positioned blade with clockwise rotation, which generated one slice per turn. Meat slices were delivered by a built-in 2-ft-long (61-cm) conveyor belt underneath the blade housing, then by an 8-ft-long (244-cm) conveyor belt attached to the meat slicer. Both slicer and conveyor belt were used in a pilot plant of a meat manufacturer before being shipped to the Center for Food Safety. Slicing speed and thickness of sliced meat were established and maintained by a specialist of Great Lakes Corporation to simulate conditions of commercial operation. Slicing conditions and thickness (approximately 2 mm) were the same for all three types of deli meat. The meat loaf was sliced and delivered continuously; however, movement of the slicer and conveyor belt were stopped periodically to collect meat slices on the 8-ft-long conveyor belt. A series of five consecutive slices of deli meat were collected, and each group of five slices was placed in a commercial film (oxygen transmission rate of 3 to 6 cm²/m²/24 h at 4.4°C) package which was then vacuum sealed (model A300/16, Multivac, Kansas City, Mo.). After enough sliced meat was collected for negative controls, 10 µl of 5% horse serum albumen containing ca. 10⁸ *L. monocytogenes* was inoculated onto a 1-in² (6.54-cm²) area of a slicing blade without removing the blade. The inoculation spot was located in the middle of the sharp edge of the blade, which had the most contact with meat during slicing. Two additional inoculation levels, ca. 10¹ and 10² CFU, were used for oven-roasted turkey studies and 10² CFU was used for a study comparing BAX-PCR and enrichment culture *L. monocytogenes* detection assays. The inoculum on the blade was air dried for 20 min at room temperature (22 to 23°C) before the blade was used. Electric fans were used to maintain airflow in the slicing room, but direct airflow to the slicer was avoided to prevent generating airborne *L. monocytogenes*. Postinoculation meat slices were collected according to the same protocol described above for the preinoculation slices. In this study, pre- and postinoculation meat packages were designated as negative controls and test samples, respectively. Thirty controls and 100 test packages were assayed on each sampling day. Depending on the sampling schedule, different numbers of control and test packages were collected. For initial studies, meat samples were assayed at 1 and 30 days postslicing, and 60 control and 200 test packages were collected. For later studies, meat samples were assayed at 1, 30, 60, and 90 days postslicing, and 120 control and 400 test packages were collected. Consecutive meat packages were distributed equally among the sampling days, e.g., packages 1, 3, 5 . . . 199 and 2, 4, 6 . . . 200 were assayed at 1 and 30 days, respectively, for 30-day trials; and packages 1, 5, 9 . . . 397, packages 2, 6, 10 . . . 398, packages 3, 7, 11 . . . 399, and packages 4, 8, 12 . . . 400 were assayed after 1, 30, 60, and 90 days, respectively, for 90-day trials. Four additional control packages were collected for chemical analyses. For studies to determine the fate of *L. monocytogenes* on meat samples, eight additional negative control packages were inoculated with *L. monocytogenes* and then vacuum sealed to serve as positive controls. Four positive control packages were inoculated.
with 100 μl of ca. 10^9 CFU in 5% horse serum albumen and the other four packages were inoculated with ca. 10^7 CFU. The 100-μl suspension was delivered in a sweeping back-and-forth motion on all five meat slices. Two positive control packages with different inoculum levels were assayed on each sampling day. All packages of sliced meat were stored at 4°C. Duplicate trials were conducted for each type of deli meat.

**Detection of L. monocytogenes in meat samples.** Detection and identification procedures were based on the USDA-FSIS Microbiology Laboratory Guidebook, 3rd edition (24), and all media and supplements were purchased from Difco, Becton Dickinson. Following up on a previous report in which L. monocytogenes was detected in more frankfurter samples when the rinse suspension of the entire contents of frankfurter packages was assayed rather than that of the standard 25-g samples (12), we assayed two sample sizes (i.e., 25 g and contents of the entire package). Because the diameter of salami was smaller than that of oven-roasted turkey and bologna, the package weight of salami was smaller than that of turkey and bologna samples. The package weight of the turkey, salami, and bologna samples ranged from 120 to 130, 75 to 80, and 120 to 130 g, respectively. For oven-roasted turkey meat sliced with the blade inoculated with 10^5 CFU listeriae and all meat samples in the study comparing the efficacy of enrichment culture and BAX-PCR assays, only the contents of the entire package were tested. To obtain 25-g samples, we stacked the five slices of deli meat in one package and cut approximately a quarter portion of the stack with a sterile knife. Each 25-g or entire-package sample was added to a stomacher bag with 225 ml of University of Vermont broth (UVM), pumped in a stomacher (model 400, Seward Ltd., Westbury, N.Y.) for 2 min at 230 rpm, and incubated at 30°C for 24 h. After incubation, 0.1 ml of enrichment culture was added to Fraser broth and a loopful of enrichment culture was streaked onto modified Oxford agar (MOX). Both MOX and Fraser broth were incubated at 37°C for 24 h. Presumptive L. monocytogenes–positive cultures in Fraser broth were streaked onto MOX and incubated at 37°C for 24 h. Presumptive L. monocytogenes–positive colonies on MOX were streaked onto horse blood overlay agar and incubated at 37°C for 24 h. Horse blood agar was prepared by using Columbia blood agar as a base and then overlaid with Columbia blood agar containing 5% horse blood. Well-isolated colonies exhibiting ß-hemolysis on horse blood overlay agar were streaked onto TSAYE, inoculated into brain heart infusion broth, and incubated at 25°C for 24 h. Colonies on TSAYE with a bluish halo under Heney illumination were confirmed as L. monocytogenes by API Listeria biochemical (bioMérieux Inc., Hazelwood, Mo.) and catalase assays. Cells in the brain heart infusion cultures were examined microscopically for cell morphology, tumbling activity, and Gram stain.

**Detection of L. monocytogenes by the BAX-PCR assay.** The BAX-PCR assay (DuPont Qualicon) for detection of L. monocytogenes (BAX–L. monocytogenes) was conducted in accordance with the instructions provided by the manufacturer. Two enrichment culture procedures were used for this assay, with the same meat-UVM homogenate described above used as the first enrichment culture. After the first incubation, 100 μl of the meat-UVM enrichment culture was transferred into 10 ml of 3-(N-morpholino)propanesulfonic acid (MOPS)–Listeria enrichment broth (MOPS-LEB) and incubated at 37°C for 24 h. Overnight cultures of L. monocytogenes H7550 and Listeria innocua in MOPS-LEB were used as positive and negative controls, respectively, for each PCR assay. Bacterial cells were lysed at 55°C for 60 min, then heated at 95°C for 10 min. After cooling to 4°C, 50 μl of lysate was added to a microtube with a tablet containing PCR reagents. The microtubes were sealed and the tablet was dissolved and placed into a 96-well plate, which was assayed by a thermocycler and a fluorescence detector. Results of PCR amplification were recorded by a computer automatically.

** Confirmatory testing of L. monocytogenes–positive samples.** Cultures in TSAYE and MOPS-LEB of L. monocytogenes–positive samples were held at 4°C until the study was terminated. For samples identified as L. monocytogenes positive only by the USDA-FSIS enrichment culture procedure, colonies on TSAYE were confirmed by the BAX-PCR assay. For samples identified as L. monocytogenes positive only by the BAX-PCR assay, cultures in MOPS-LEB were streaked onto MOX, incubated at 37°C for 24 h, then assayed for L. monocytogenes using the USDA-FSIS enrichment culture procedure described above.

**Enumeration of L. monocytogenes.** A three-tube most-probable-number (MPN) procedure was used to determine L. monocytogenes cell numbers in meat samples in accordance with the protocol described in the USDA-FSIS Microbiology Laboratory Guidebook. Enumeration assays were only conducted on L. monocytogenes–positive samples, including L. monocytogenes–positive controls in the comparison study of BAX-PCR and USDA-FSIS enrichment culture assays. Thirty-five milliliters of the meat-UVM homogenate was stored at 2°C immediately after stomaching. Based on previous results, L. monocytogenes counts did not substantially change when samples were held at this temperature for 5 days (unpublished data). When L. monocytogenes was detected in meat samples, MPN assays were done to determine L. monocytogenes cell numbers. The 30-ml homogenate was aliquoted into three tubes, then twice serially diluted (1:10) with UVM, and all tubes were incubated at 37°C for 24 h. MPN determinations of L. monocytogenes–positive control samples were conducted simultaneously with enrichment culture. A direct plating procedure was used for L. monocytogenes–positive control samples of turkey meat held for 60 and 90 days postslicing because high counts of L. monocytogenes were anticipated. For those samples, meat-UVM homogenate was serially diluted (1:10) in Butterfield’s phosphate buffer and plated onto duplicate MOX plates. Colonies with characteristics typical of L. monocytogenes on MOX plates were confirmed by BAX-PCR assay.

**Equipment surface sampling.** Twenty locations on the slicer and associated equipment that contacted meat slices and sliced meat debris were selected for L. monocytogenes testing. These included 10 sites on surfaces underneath the blade housing of the slicer, 6 sites on the blade, and 4 sites on conveyor belt surfaces (Table 1). After collecting all meat slices, each location was swabbed with a sterile sponge (Whirl-Pak, Nasco, Fort Atkinson, Wis.). Ten milliliters of Dey-Engley neutralizing broth was added to each prepackaged sponge before swabbing. One hundred milliliters of UVM was added to each package with the sponge after swabbing. The same procedures as described above were used to isolate and identify L. monocytogenes from cultures of equipment swabs.

**Cleaning and sanitation of the slicer.** After completion of slicing and environmental sampling, meat debris was removed by paper towels saturated with 70% ethanol. The slicer and conveyor belt were sprayed with 70% ethanol, air dried for 15 to 20 min, and sprayed with hot water (50 to 55°C). The slicer was disassembled, and the removable parts and slicer were scrubbed and treated with alkali sanitizer (FOAMATE, Zep Co. Atlanta, Ga.) at the manufacturer’s recommended concentration, then rinsed with hot water. One day before slicing, all parts of the slicer and conveyor belts were rinsed with hot water, air dried, sprayed with...
TABLE 1. Sampling sites and locations of equipment surface sampling on the slicer and conveyor belts

<table>
<thead>
<tr>
<th>Sampling site no.</th>
<th>Locations</th>
<th>Area sampled (cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1–4</td>
<td>Bottom layer of surface underneath blade housing</td>
<td>413</td>
</tr>
<tr>
<td>5 and 6</td>
<td>Rear panels of area underneath blade housing</td>
<td>748</td>
</tr>
<tr>
<td>7–10</td>
<td>Side panels of area underneath blade housing</td>
<td>767</td>
</tr>
<tr>
<td>11 and 12</td>
<td>Side panels of 2-ft conveyor belt</td>
<td>74</td>
</tr>
<tr>
<td>13</td>
<td>Hole beside 2-ft conveyor belt</td>
<td>305</td>
</tr>
<tr>
<td>14</td>
<td>Surface of 2-ft conveyor belt</td>
<td>1,539</td>
</tr>
<tr>
<td>15</td>
<td>Surface of 8-ft conveyor belt</td>
<td>3,755</td>
</tr>
<tr>
<td>16</td>
<td>Top surface of blade housing</td>
<td>2,919</td>
</tr>
<tr>
<td>17</td>
<td>Bottom surface of blade housing</td>
<td>2,819</td>
</tr>
<tr>
<td>18</td>
<td>Surface of blade (including the inoculation spot)</td>
<td>1,406</td>
</tr>
<tr>
<td>19</td>
<td>Side surface of blade housing</td>
<td>243</td>
</tr>
<tr>
<td>20</td>
<td>Hole beside the blade on the blade housing</td>
<td>6.4</td>
</tr>
</tbody>
</table>

70% ethanol, air dried, and assembled. The blade was sanitized as described previously, sharpened, and attached to the slicer on the day before each experiment.

Chemical and pH analyses of deli meats. Water activity, pH, moisture content, fat content, and salt content of deli meat were determined according to procedures of the AOAC International (1). Total moisture, fat, and salt contents were determined as weight percentage (water, fat, or salt weight/total sample weight × 100). Deli meat was ground by a food processor (Black & Decker, Aplica Consumer Products, Miami Lakes, Fla.) for 30 to 45 s for water activity, moisture content, and salt content determinations. Water activity was determined on 2-g ground samples with a water activity measurement device (model CX2, Aqua Lab, Pullman, Wash.) in a temperature-controlled room at 21°C. Moisture content was determined on 2-g ground sample by drying in a vacuum oven (model 280A, Fisher Scientific) at 100°C for 24 h and calculating the difference of weight before and after drying. Fat content was determined on 2-g meat samples sliced into pieces by a knife. Samples were dried in a vacuum oven and hydrolyzed in boiling 16% HCl for 5 min, and total fat was extracted with a solution of ether and petroleum ether, which in a beaker was subsequently evaporated in a fume hood for 16 to 18 h and further dried in a vacuum oven (model 280A Fisher Scientific) at 70°C for 6 h. Each beaker was cooled in a desiccator and weighed. Fat content of the meat sample was determined by weight difference of the beaker with the dried solvent extract and the empty beaker. Salt content was determined from 4-g ground meat samples homogenized in 50 ml of hot water for 2 min at ca. 55°C by a Polytron (model PCU 11, Kinematica AG, New Haven, Conn.). Two to 3 ml of 17.5% HNO₃ was added to the homogenate and titrated with 0.085 N silver nitrate using a combination three-in-one pH electrode (model 476436, Corning Corporation, Corning, N.Y.) and silver billet electrode (Beckman Coulter, Fullerton, Calif.) connected to a pH meter (model 350, Corning Corporation). pH values were obtained by placing a flat-surface pH probe (model 450C, SensoreX, Garden Grove, Calif.) onto the meat surface. Duplicate samples of each type of deli meat were used for each analysis, and two analyses of each sample were performed for each chemical and pH analysis.

Statistical analysis. Results of duplicate tests were combined and significant difference (P = 0.05) in sampling size (25 g versus entire package) or detection methods (enrichment culture versus PCR) was determined by t test for paired samples. All statistical analyses were done with the Statistical Analysis System program (SAS, Cary, N.C.).

RESULTS

Chemical characteristics of deli meats. Among the three types of deli meat, salami had the lowest pH (5.0) and moisture content (44%) and the highest fat (32.6%) and sodium chloride (4.0%) contents (Table 2). Oven-roasted turkey had the lowest sodium chloride (1.1%) and fat (4.6%) contents and the highest moisture content (74.6%). Fat contents of bologna (29.5%) and salami (32.6%) were similar, as were the pH values of bologna (6.09) and oven-roasted turkey (6.33).

Detection of L. monocytogenes on deli meats. Among the three types of deli meat, the greatest number of L. monocytogenes–positive samples was obtained from oven-roasted turkey after storage at 4°C (Tables 3 and 4). Using larger cell numbers of listeriae for inoculation of the slicer blade resulted in a greater number of L. monocytogenes–positive turkey samples. In addition, the number of L. monocytogenes–positive turkey samples increased during storage for all inoculation levels, whereas L. monocytogenes–positive salami and bologna samples decreased (Tables 3 and 4). Increasing the sample size from 25 g to the contents of the entire package resulted in slightly improved detection of L. monocytogenes on oven-roasted turkey (12 versus 14) at 1 day postslicing. Twice as many L. monocytogenes–positive samples (36 versus 18, P < 0.05) of turkey meat were detected at 30 days postslicing when 10⁵ CFU were used to inoculate the blade and contents of the entire package compared with a 25-g sample (Table 3). However, a larger sample size did not yield an increased number of L. monocytogenes–positive samples for salami and bologna (Table

TABLE 2. Chemical characteristics and pH of oven-roasted turkey, salami, and bologna

<table>
<thead>
<tr>
<th>Deli meat</th>
<th>pH</th>
<th>Water activity</th>
<th>Moisture (%)</th>
<th>Fat (%)</th>
<th>Sodium chloride (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Turkey</td>
<td>6.33 ± 0.35</td>
<td>0.96 ± 0.11</td>
<td>74.6 ± 2.1</td>
<td>4.6 ± 0.3</td>
<td>1.1 ± 0.0</td>
</tr>
<tr>
<td>Salami</td>
<td>5.03 ± 0.26</td>
<td>0.91 ± 0.08</td>
<td>44.0 ± 1.8</td>
<td>32.6 ± 1.1</td>
<td>4.0 ± 0.1</td>
</tr>
<tr>
<td>Bologna</td>
<td>6.09 ± 0.36</td>
<td>0.93 ± 0.05</td>
<td>50.6 ± 2.1</td>
<td>29.5 ± 0.7</td>
<td>2.0 ± 0.1</td>
</tr>
</tbody>
</table>

* Results are the average ± standard deviation of two analyses for each chemical analysis of each type of deli meat, and duplicate samples were used for each analysis (wt/wt basis).
TABLE 3. L. monocytogenes–positive equipment surface and meat samples at 1 and 30 days postslicing when sliced by a blade inoculated with ca. $10^3$ CFU of L. monocytogenes

<table>
<thead>
<tr>
<th>Deli meat</th>
<th>Equipment samples</th>
<th>Day 1</th>
<th>Day 30</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>25 g</td>
<td>Entire package</td>
</tr>
<tr>
<td>Turkey</td>
<td>3/80</td>
<td>12/200 A</td>
<td>14/200 A</td>
</tr>
<tr>
<td>Salami</td>
<td>7/80</td>
<td>7/200 A</td>
<td>2/200 b</td>
</tr>
<tr>
<td>Bologna</td>
<td>1/80</td>
<td>1/200 A</td>
<td>1/200 A</td>
</tr>
</tbody>
</table>

a Samples of 25 g and contents of entire packages were not obtained from the same slicing trial. Duplicate trials were conducted for each sample size and each type of deli meat. Results are the total number of L. monocytogenes–positive samples from two trials for each sample size. Meat samples were held at 4°C following slicing.

b Weight of the entire package of turkey, salami, and bologna was 120 to 130, 75 to 80, and 120 to 130 g, respectively.

c Numbers followed by different letters in the same row on the same sampling day were significantly different ($P = 0.05$).

When low L. monocytogenes inoculation levels ($10^1$ or $10^2$ CFU) were used, no positive samples were detected at 1 and 30 days postslicing (Table 4). At the $10^3$ CFU inoculation level, three and five L. monocytogenes–positive turkey samples were detected at 60 and 90 days postslicing, respectively, by assaying the contents of the entire package, but no listeriae were detected on the same sampling days by assaying 25-g samples (Table 4), which is the reason for assaying only the contents of the entire package for studies with an inoculum level of $10^2$ CFU and in the later study comparing BAX-PCR and enrichment culture assays (Table 5). At the $10^2$ CFU inoculation level, 7 and 33 L. monocytogenes–positive samples were detected at 60 and 90 days postslicing, respectively (Table 4).

Efficacy of detecting L. monocytogenes by the USDA enrichment culture and BAX-PCR assays. L. monocytogenes was detected in 12 meat samples by both the USDA enrichment culture and BAX-PCR procedures (Table 5). However, L. monocytogenes was detected in eight samples only by the BAX-PCR and not by the enrichment culture assay, with a significant difference ($P < 0.05$) occurring for the 90-day samples of oven-roasted turkey. L. monocytogenes was not detected on two positive control meat samples inoculated at the $10^3$ CFU level by either enrichment culture or BAX-PCR assays but was detected by the MPN procedure. Colonies on TSYE plates from samples in which L. monocytogenes was detected by the USDA enrichment culture assay were all confirmed as L. monocytogenes positive by the BAX-PCR assay. However, for two samples in which L. monocytogenes was detected only by the BAX-PCR assay, the pathogen was not detected in the corresponding MOPS-LEB broth by the USDA enrichment culture procedure.

The number of L. monocytogenes–positive samples of salami and bologna decreased during storage, with L. monocytogenes not detectable on positive controls at 60 or 90 days postslicing on either salami or bologna. In contrast, the greatest number of L. monocytogenes–positive turkey meat samples were detected at 90 days postslicing. Throughout the entire study, L. monocytogenes was not detected on any negative control samples that were not inoculated with listeriae.

L. monocytogenes cell numbers on deli meat. L. monocytogenes cell numbers on the positive samples detected by comparing BAX-PCR and USDA enrichment culture assays were generally very low (<0.2 MPN/g) when detected on deli meats contaminated by the slicer and held at 4°C for up to 90 days (Tables 6 through 8). Cell numbers of L. monocytogenes could be determined only by the MPN procedures, except for the positive control samples of turkey meat stored for 60 and 90 days postslicing. L. monocytogenes cell numbers on some positive control samples could not be determined by the MPN procedure. For these samples, L. monocytogenes cell numbers were reported as less than 0.03 MPN/g, the lowest number in the MPN table. L. monocytogenes cell numbers decreased and eventually became nondetectable on salami and bologna during storage;
TABLE 5. Number of L. monocytogenes-positive meat samples of entire contents of packages determined by the USDA enrichment culture or BAX-PCR assays after storage at 4°C

<table>
<thead>
<tr>
<th>Deli meat&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Day</th>
<th>Equipment samples</th>
<th>Meat samples</th>
<th>Positive controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Culture</td>
<td>PCR</td>
<td>Culture</td>
</tr>
<tr>
<td>Turkey</td>
<td>1</td>
<td>1/40</td>
<td>1/40</td>
<td>2/200</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>0/200</td>
<td>0/200</td>
<td>0/200</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>1/200</td>
<td>3/200</td>
<td>3/4&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>2/200&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5/200&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4/4</td>
</tr>
<tr>
<td>Salami</td>
<td>1</td>
<td>0/40</td>
<td>0/40</td>
<td>1/200</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>0/200</td>
<td>0/200</td>
<td>0/200</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>0/200</td>
<td>0/200</td>
<td>0/4</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>1/200</td>
<td>1/200</td>
<td>0/4</td>
</tr>
<tr>
<td>Bologna</td>
<td>1</td>
<td>1/40</td>
<td>1/40</td>
<td>2/300</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>1/300&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1/300&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4/6&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>1/300&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1/300&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4/6&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>1/300&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1/300&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4/6&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Results for bologna were the combined total number of L. monocytogenes-positive samples for three trials. Results for salami and oven-roasted turkey were the combined total number of L. monocytogenes-positive samples for two trials.

<sup>b</sup> Only the results from turkey on day 90 were significantly different (P < 0.05) between enrichment culture and PCR assays.

<sup>c</sup> L. monocytogenes was not detectable in some positive control samples inoculated with 10<sup>0</sup> CFU.

<sup>d</sup> L. monocytogenes was detected in only one sample inoculated with 10<sup>1</sup> CFU in the second trial.

<sup>e</sup> The corresponding MOPS-LEB broth of the two positive samples was determined as negative by the USDA enrichment culture method.

<sup>f</sup> Positive samples were detected only for the second trial, in which temperature abuse occurred.

However, L. monocytogenes cell numbers increased with time on turkey meat (Table 6).

Between 60 and 90 days postslicing of the second trial of bologna, the storage temperature increased to 16°C for three days due to a power outage. Hence, a third trial of bologna was conducted. There were no L. monocytogenes-positive samples for the first and third trials at 90 day postslicing; however, L. monocytogenes was detected at 90 days postslicing in the second trial (Table 5). In addition, no L. monocytogenes was detected in the positive control bologna samples of the first and third trials at 90 days postslicing but was detected in both positive control samples at 90 days postslicing in the second trial. High MPN cell numbers, 5 and 50 MPN/g, were detected in these two positive controls. However, L. monocytogenes cell numbers were low, less than 0.03 MPN/g, on the L. monocytogenes-positive slicer-contaminated samples of the second trial (Table 8).

Detection of L. monocytogenes on processing equipment. When the slicer blade was inoculated with 10<sup>3</sup> CFU L. monocytogenes, listeriae were detected on three, eight,

TABLE 6. L. monocytogenes counts or most probable numbers on oven-roasted turkey at different sampling times<sup>a</sup>

<table>
<thead>
<tr>
<th>Day</th>
<th>Equipment samples</th>
<th>Meat samples</th>
<th>Positive controls&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Culture</td>
<td>PCR</td>
<td>Culture</td>
</tr>
<tr>
<td>Trial 1</td>
<td>1</td>
<td>ND&lt;sup&gt;c&lt;/sup&gt;</td>
<td>&lt;0.03</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>ND</td>
<td>0.075&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>0.15</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>0.19, &lt;0.03&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0.19</td>
</tr>
<tr>
<td>Trial 2</td>
<td>1</td>
<td>&lt;0.03&lt;sup&gt;g&lt;/sup&gt;</td>
<td>&lt;0.03</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>ND</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>&lt;0.03</td>
<td>0.26</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>&lt;0.03&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0.75</td>
</tr>
</tbody>
</table>

<sup>a</sup> Values are CFU per gram or MPN per gram, respectively.

<sup>b</sup> Two inoculation levels, 10<sup>0</sup> and 10<sup>1</sup> CFU per package, were used.

<sup>c</sup> ND, not detectable.

<sup>d</sup> Sample was detected by MPN only, not by enrichment culture or BAX-PCR procedures.

<sup>e</sup> Direct plating (CFU per gram) was used to determine L. monocytogenes counts.

<sup>f</sup> There were three and four L. monocytogenes-positive samples for the first and second trial, respectively. L. monocytogenes cell numbers on all positive samples, except one, were <0.03 MPN/g.

<sup>g</sup> L. monocytogenes cell numbers on equipment samples were determined as MPN per milliliter of enrichment culture.

TABLE 7. L. monocytogenes counts or most probable numbers on salami at different sampling times

<table>
<thead>
<tr>
<th>Day</th>
<th>Equipment samples</th>
<th>Meat samples</th>
<th>Positive controls&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Culture</td>
<td>PCR</td>
<td>Culture</td>
</tr>
<tr>
<td>Trial 1</td>
<td>1</td>
<td>ND&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Trial 2</td>
<td>1</td>
<td>ND&lt;sup&gt;c&lt;/sup&gt;</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>0.15</td>
<td>ND</td>
</tr>
</tbody>
</table>

<sup>a</sup> Values are CFU per gram or MPN per gram, respectively.

<sup>b</sup> Two inoculation levels, 10<sup>0</sup> and 10<sup>1</sup> CFU per package, were used.

<sup>c</sup> ND, not detectable.
and one equipment surface samples after slicing turkey, salami, and bologna, respectively (Table 3). With low inoculum levels at $10^0$ and $10^1$ CFU per package, were used.

$L. monocytogenes$ cell numbers on equipment samples were determined as MPN per milliliter of enrichment culture.

ND, not detectable.

Temperature (3 days at 16°C) occurred between 60 and 90 days postslicing.

$L. monocytogenes$ cell numbers in both positive samples were $<0.03$ MPN/g.

$L. monocytogenes$ was detected by MPN procedure only, not by enrichment culture or BAX-PCR procedures.

and one equipment surface samples after slicing turkey, salami, and bologna, respectively (Table 3). With low inoculum levels at $10^0$ and $10^1$ CFU per package, $L. monocytogenes$ was not detected on any equipment surface samples (Table 4). In comparing the BAX-PCR and enrichment culture assays, the number of $L. monocytogenes$–positive equipment samples were one, zero, and one after slicing turkey, salami, and bologna, respectively (Table 5). All $L. monocytogenes$–positive equipment samples in this comparison study were detected with both the PCR and enrichment culture assays.

$L. monocytogenes$ cell numbers were $<0.03$ and 0.36 MPN/g for the positive samples after slicing turkey and bologna, respectively. A layer of fat was observed on the slicer blade and conveyor belts after slicing the salami, and a layer of clear liquid was observed after slicing the turkey meat. Numbers of $L. monocytogenes$–positive equipment surface samples were five, four, two, and two for blade surface, blade housing, 2-ft conveyor belt, and left inside corner of the area underneath the blade housing, respectively.

**DISCUSSION**

Adherence and survival of $L. monocytogenes$ on the surface of commercial processing equipment have been well studied; however, the dynamics of cross-contamination have not been reported. Our study revealed that $L. monocytogenes$ could be transferred from a commercial slicer and possibly associated equipment onto deli meat, and the degree of transfer was correlated with the number of listeriae inoculated onto the slicer blade. $L. monocytogenes$ cell numbers present on contaminated processing equipment of commercial facilities are believed to be low in most situations because $L. monocytogenes$ is typically not detected by direct plating but rather only by enrichment culture (16, 17). A recent survey of retail luncheon meats revealed contamination levels for 99.6% of samples of less than one $L. monocytogenes$ per gram (8). Hence, $10^3$ CFU of $L. monocytogenes$ is likely to be a worst case scenario for contamination of a commercial slicer.

Fewer $L. monocytogenes$–positive samples were obtained from salami and bologna containing lactate and diacetate than from oven-roasted turkey following many days of refrigerated storage. These results are in agreement with previous studies in which $L. monocytogenes$ grew rapidly on sliced poultry products but slowly or were inhibited on fermented meat products such as salami (5, 9), which had a lower moisture content and pH value than turkey meat. In addition, although beef bologna had a pH similar to oven-roasted turkey, the presence of sodium lactate and potassium diacetate in the bologna inhibited growth of $L. monocytogenes$ (14, 20, 23). Results from the $L. monocytogenes$–inoculated positive control samples in our study revealed that $L. monocytogenes$ cell numbers increased on turkey meat but decreased on salami and bologna during refrigerated storage. However, $L. monocytogenes$ cell numbers increased on bologna with preservatives at elevated temperature. Therefore, storage temperature is an important factor to controlling $L. monocytogenes$ when lactate and diacetate are added as preservatives.

Two more turkey meat samples were $L. monocytogenes$ positive at 1 day postslicing when the contents of the entire package rather than a 25-g sample (14 versus 12, respectively) were assayed. However, twice the number of $L. monocytogenes$–positive samples were obtained at 30 days postslicing by assaying the contents of the entire package rather than a 25-g sample (36 versus 18, respectively). $L. monocytogenes$ cell numbers are likely a contributing factor to increased findings of $L. monocytogenes$ on turkey meat because a substantially larger sample size (i.e., 120 to 130 g) likely contains more listeriae than a 25-g sample. An additional contributing factor may be that listeriae did not cover the entire meat slice, with colonies of listeriae growing in localized areas of contamination. Hence, *Listeria* may not be present in a 25-g sample but is elsewhere on the meat within the package, thereby yielding positive results from the larger sample assayed. For bologna, very few $L. monocytogenes$–positive samples were obtained at 1 day postslicing with either sample size (one versus one), and $L. monocytogenes$ cell numbers decreased during storage. Therefore, the influence of sample size to detect $L. monocytogenes$ in bologna could not be determined. In contrast, a greater number of *Listeria*-positive samples were detected in the 25-g samples of salami than in the contents of the entire package at 1 day postslicing (seven versus two). A low dilution ratio of broth to meat by assaying the contents of the entire package was determined not to be a factor for recovering $L. monocytogenes$ based on results of a preliminary study and the $L. monocytogenes$–positive control samples. However, because a relatively small number of

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**TABLE 8.** $L. monocytogenes$ counts or most probable numbers on bologna at different sampling times

| Day | Equipment samples | Meat samples | Positive controls
|-----|------------------|--------------|---------------------|
|     |                  |              | $10^0$ | $10^1$
| Trial 1 | 1 | 0.036$^c$ | ND$^d$ | ND | 0.75 | 30 | <0.03 | ND | <0.03 | 60 | ND | <0.03 | 1.23 | 90 | ND | ND |
| Trial 2$^e$ | 1 | ND | <0.03$^f$ | ND | 1.04 | 30 | <0.03 | 0.15 | 0.19 | 60 | <0.03 | ND | 0.19 | 90 | <0.03 | 5 | 50 |
| Trial 3 | 1 | ND | ND | 0.1$^g$ | 0.265 | 30 | ND | 0.15 | 0.265 | 60 | ND | ND | 0.1 | 90 | ND | ND |

$^a$ Values are CFU per gram or MPN per gram, respectively.

$^b$ Two inoculation levels, $10^0$ and $10^1$ CFU per package, were used.

$^c$ $L. monocytogenes$ cell numbers on equipment samples were determined as MPN per milliliter of enrichment culture.

$^d$ ND, not detectable.

$^e$ Temperature (3 days at 16°C) occurred between 60 and 90 days postslicing.

$^f$ $L. monocytogenes$ cell numbers in both positive samples were $<0.03$ MPN/g.

$^g$ $L. monocytogenes$ was detected by MPN procedure only, not by enrichment culture or BAX-PCR procedures.
seven and two positive samples from 200 meat packages were detected in this study, an additional study using a larger number of samples would be beneficial to validate the influence of sample size on Listeria detection in salami and bologna samples. Studies by Luchansky et al. (12) revealed higher rates of detection of L. monocytogenes in frankfurters by enrichment culture of the rinse fluid of the contents of the entire package than by a macerate of a 25-g sample.

Although most L. monocytogenes–positive samples were obtained from meat packages that were among the first to be sliced, they were also obtained from meat packages sampled at the middle or near the end of slicing. This was not a surprising finding because L. monocytogenes has been reported to adhere to equipment such as a dicing machine for an extended period of time and even be carried from one manufacturing facility to another (11). Hence, L. monocytogenes likely adhered to the slicer blade and housing, and possibly the conveyor belt, during the slicing operation, thereby contributing to contamination of meat at the middle or later stages of slicing. The considerably higher incidence of listeriae on equipment surfaces after slicing salami than the other deli meats is likely due to the large amount of fat from the salami that covered the equipment following slicing. This excessive smearing of fat on equipment surfaces did not occur with the turkey meat or bologna. The location of Listeria contamination was likely affected by design of the slicer. The direction in which the blade rotated resulted in an accumulation of most meat debris in the left inside corner of the slicer housing and was the only section underneath the blade housing having L. monocytogenes–positive swab samples. A small hole on the side of the short (61 cm) conveyor belt was another location where meat debris accumulated during slicing and swab samples from this area were frequently L. monocytogenes positive. Newer models of slicers have been redesigned to eliminate such hard-to-clean areas as the hole associated with the conveyor belt (personal communication, specialists of Great Lakes Corporation). Our results reinforce the importance of cleaning and sanitation of slicing equipment in preventing cross-contamination by listeriae. Based on our Listeria–negative results (data not shown) of equipment swab samples before slicing and negative control samples, proper cleaning and sanitation can prevent L. monocytogenes from a previously contaminated slicer. It should be noted that the slicing operation in this study was different from that typically used in an industrial operation in that the amount of meat sliced and operation time were considerably less than occur in a commercial operation, and there were at least 2-week gaps between each slicing trial.

Detection efficacy of the BAX-PCR assay for L. monocytogenes in deli meat has been reported previously to be equivalent to or better than the enrichment culture procedure (10, 11, 22). Results of one study revealed that the two assays were equivalent when L. monocytogenes was present at 10 to 50 CFU/25 g, but more positive samples were detected by the BAX-PCR than by enrichment culture procedure (30 versus 17), with L. monocytogenes cell numbers of 1 to 5 CFU/25 g (10). In our study, more positive samples were detected by the BAX-PCR than by enrichment culture assays in the L. monocytogenes–positive control samples inoculated at lower level of inoculum, 10^0 CFU, and the 90-day turkey samples.

L. monocytogenes cell numbers in positive control samples were low, and growth of L. monocytogenes on inoculated positive controls of turkey meat was considerably greater than on positive samples contaminated by slicing. Perhaps the slicing event by a commercial slicer injures listeriae, thereby affecting their recovery and growth on contaminated sliced meats.

In summary, contamination of deli meat during slicing was correlated with the number of L. monocytogenes initially on a slicer blade and the type of meat being sliced, with more L. monocytogenes–positive samples obtained from oven-roasted turkey than from beef bologna and salami. Greater numbers of L. monocytogenes–positive samples were obtained by using a larger sample size of turkey meat. Therefore, the degree of transfer of L. monocytogenes to sliced meats is product dependent as is the ideal sample size for detecting L. monocytogenes in sliced meats. The BAX-PCR assay appeared to be an acceptable alternative to the USDA enrichment culture procedure for detecting L. monocytogenes in precooked deli meat.

ACKNOWLEDGMENTS

We thank Bobby Goss, David Mann, Josh Nichols, Clint Patton, Hoi-Kyung Kim, and Lynne Thurber for technical assistance.

REFERENCES


May 3, 2007

Division of Dockets Management (HFA-305)
Food and Drug Administration
5630 Fishers Lane, R. 1061
Rockville, MD  20852


To Whom It May Concern:

The American Meat Institute (AMI) is the nation's oldest and largest meat packing and processing industry trade association. Our members slaughter and process over 90 percent of the nation's beef, pork, lamb, veal and nearly 75 percent of the turkey produced in the United States. AMI appreciates the opportunity to comment on the U.S. Food and Drug Administration (FDA) “Draft Documents on the Safety of Animal Clones”.1

AMI and our member companies recognize the success of our industry rests upon our ability to produce safe, wholesome, consistent and high quality products. New technologies play a role in the industry’s ability to make continuous improvements in food production. The use of Advanced Reproductive Technologies, (ARTs) have a history of enabling improvements in livestock production and we agree with FDA that Somatic Cell Nuclear Transfer (cloning) falls upon the continuum of ARTs currently used in animal agriculture. Cloning will provide farmers and ranchers another alternative for breeding the most productive, healthiest possible animals, which will enhance the reliable production of safe and healthy food.

We applaud FDA for the thorough process taken to develop and seek comments on the cloning risk assessment, proposed risk management plan, and the guidance to industry. We support the agency’s transparent dialogue with stakeholders, including the international scientific community, and encourage the agency to continue this important transparent dialogue with stakeholders in the U.S. and internationally. AMI also supports the FDA’s continued voluntary moratorium on products from animals produced through cloning as well as the offspring of clones while the draft documents are in the public comment and finalization stage.

Product Safety and Animal Health

AMI supports the conclusions of the Draft Animal Cloning Risk Assessment by the FDA which finds meat and milk from cloned animals and their offspring to be as safe to eat as corresponding products from animals produced using other ARTs and natural mating. We find that the extensive time (over five years) that FDA spent reviewing and analyzing hundreds of peer-reviewed publications, in addition to assessing a large quantity of analytical data from products of clones speaks to the scientific thoroughness and validity of the conclusions drawn on the safety of these products. In addition, having the National Academies of Sciences (2002)\(^2\) conduct an independent data collection and review of cloning technology and its possible risks further bolsters the scientific credibility of the FDA conclusions. FDA should be commended on the transparency of the process, making documents available to the public and hosting a meeting to seek public comments.

AMI agrees with FDA that the risks to animal health presented by cloning are within the range of what would be expected to occur with other ARTs that have been in use for many years.

Labeling

Meat and milk from cloned animals and their progeny have been documented to be indistinguishable from food from animals bred using other ARTs. Given this fact, and following the requirement set by FDA that food labels must be “truthful and not misleading” to consumers, we support FDA’s current position to not require labeling for meat or meat products from animals derived from cloned animals and their progeny. Furthermore, since the products are indistinguishable from the comparators there are no new “material facts” that would require disclosure to consumers. Labeling would create a false impression among consumers that products resulting from clones and their progeny were somehow different when clearly the scientific data reviewed by the FDA demonstrate there are not discernible differences. Therefore, because the FDA has concluded that meat and milk from cloned animals and their offspring are no different than products from their conventional counterparts, we expect and support that the FDA will not require labeling of these food products.

Stakeholder Information

AMI recognizes that consumer acceptance of products that are the result of new technologies, such as ARTs, is critical to technology acceptance and adoption by

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\(^2\) 2002 Animal Biotechnology: Science-Based Concerns.
http://www.nap.edu/books/0309084393/html/
industry. AMI recognizes that concerns raised about the use of cloning have the potential for negative economic impact to the meat industry. In this regard, we encourage the FDA to continue to inform stakeholders about the scientific basis for animal health and product safety of products derived from ARTs including cloning and FDA should be a partner with stakeholders in addressing concerns that have been raised about cloning. As with any new technology, the commercial market will ultimately decide if and when it may be adopted; but it is important that those market decisions be based upon a solid scientific foundation of knowledge and facts. We encourage FDA to continue to monitor new research findings relative to cloning in the U.S. and international scientific communities and to convey any new information to key stakeholder groups as it becomes available.

In summary, AMI believes that encouragement of new technological advancements in animal agriculture is important to keeping U.S. producers and processors competitive in domestic and world markets. We appreciate FDA’s thorough scientific review of this new technology and support the FDA’s commitment to continued surveillance of developments associated with this technology.

We appreciate the opportunity to comment on this important initiative within the agency.

Respectfully submitted,

Randall D. Huffman, Ph.D.
Vice President, Scientific Affairs
American Meat Institute Foundation

cc: J. Patrick Boyle
    Mark Dopp
    Jim Hodges
AMIF Research Update
# AMIF Research Proposals Approved for Funding

## Ranked in Priority by AMIF Research Advisory Committee

<table>
<thead>
<tr>
<th>Proposal #</th>
<th>Investigator(s)</th>
<th>Organization(s)</th>
<th>Project Title</th>
<th>Cost &amp; Timeline</th>
</tr>
</thead>
<tbody>
<tr>
<td>06-138</td>
<td>Chance Brooks, Mindy Brashears, Mark Miller, Adam Tittor</td>
<td>Texas Tech University</td>
<td>Impact of Ground Beef Packaging Systems and Temperature Abuse on the Safety and Quality of Ground Beef</td>
<td>$63,245&lt;sup&gt;1&lt;/sup&gt; One year</td>
</tr>
<tr>
<td>06-407</td>
<td>Jeffrey Savell, Kerri Harris, Alejandro Castillo, Wesley Osburn</td>
<td>Texas A&amp;M University</td>
<td>Evaluation of Alternative Cooking and Cooling Procedures for Large, Intact Meat Products to Achieve Lethality and Stabilization Microbiological Performance Standards</td>
<td>$74,500 One year</td>
</tr>
<tr>
<td>06-406</td>
<td>Randy Wheling, Michael Zeece, Harshavardhan Thippareddi</td>
<td>University of Nebraska</td>
<td>Evaluation and Analysis of Meat Products Contaminated by Low Levels of Ammonia</td>
<td>$67,350 Two years</td>
</tr>
<tr>
<td>06-403</td>
<td>M. Ellin Doyle, Ronald Weiss, Stacey Schultz-Cherry, Hon Ip, Michael Robach</td>
<td>University of Wisconsin, USGS National Wildlife Health Center, Cargill, Inc.</td>
<td>White Paper on Destruction of H5N1 Avian Influenza in Meat and Poultry Products</td>
<td>$10,000 Five months</td>
</tr>
<tr>
<td>06-201</td>
<td>Charles Carpenter, Jeffrey Broadbent</td>
<td>Utah State University</td>
<td>Validation of Levulinic Acid for Topical Decontamination of Meat Surfaces</td>
<td>$63,263 Two years</td>
</tr>
<tr>
<td>06-317</td>
<td>Annette O’Connor</td>
<td>Iowa State University</td>
<td>A Systematic Review of Literature on Pork Chain Epidemiology</td>
<td>$33,215&lt;sup&gt;2&lt;/sup&gt; Two years</td>
</tr>
<tr>
<td>06-411</td>
<td>Randall Phebus, Douglas Powell, Harshavardhan Thippareddi</td>
<td>Kansas State University, University of Nebraska</td>
<td>Beyond Intent: Assessment and Validation of On-package Handling and Cooking Instructions for Uncooked, Breaded Meat and Poultry Products to Promote Consumer Practices that Reduce Foodborne Illness Risks</td>
<td>$95,000 Two years</td>
</tr>
</tbody>
</table>

**Total Funds Requested: $406,573**

<sup>1</sup> The total project cost is $126,490. NCBA has agreed to co-fund this research.

<sup>2</sup> The total project cost is $66,429. The National Pork Board has agreed to co-fund this research.
Research Project Status Report

Project: 06-138

Investigators: Chance Brooks, Mindy Brashears, Mark Miller, Adam Tittor

Organization: Texas Tech University

Title: Impact of Ground Beef Packaging Systems and Temperature Abuse on the Safety and Quality of Ground Beef

Cost: $63,245 (Total project cost is $126,490. NCBA is co-funding this research)

Timeline: One year

End Date: March 2008

Description: The objectives of this study are to: 1. Determine the impact of packaging methods and temperature abuse on the pathogen loads in ground beef inoculated with *E. coli* O157 and *Salmonella*; and 2. Determine the effect of packaging methods and temperature abuse on the spoilage characteristics and shelf life of ground beef.

These objectives will be accomplished with two studies – a pathogen study conducted at the BSL II Food Safety Laboratory at Texas Tech University-Experimental Sciences Building and a shelf life study conducted at the GW Davis Meat Science Laboratory. Coarse-ground beef will be obtained from a commercial processor located 45 miles from Lubbock, TX. Ground beef patties will be produced and allotted randomly to five packaging treatments. The five packaging treatments evaluated will be: 1) control (foam tray with film over-wrap); 2) high-oxygen (80% O₂ / 20% CO₂) modified atmosphere package (MAP); 3) low-oxygen MAP without carbon monoxide; 4) low-oxygen carbon monoxide (0.4% CO, 30% CO₂, 69.6% N₂) MAP; and 5) vacuum (clear pouch/bag). Packaged patties will be stored in the dark for 5 days, displayed under retail lighting for 5 days at abusive temperatures, transferred to additional retail display cases maintained at 0°C and displayed for up to 10 days. Samples will be removed from the test at regular intervals throughout the study to evaluate the effects of packaging treatment on pathogen levels or spoilage characteristics.

The project will generate information, which can be used for educational purposes, to inform the industry about ways to control *Escherichia coli* O157:H7 and *Salmonella* in fresh meat products. Finally, the information generated from this project can be used by AMIF to educate law-makers and regulatory personnel with scientifically-validated data pertaining to the safety and spoilage characteristics of carbon monoxide as a packaging environment.

For the complete research description please contact Susan Backus at sbackus@meatami.com

May 2007
| Status | AMIF and NCBA have entered into research agreements with Texas Tech University. Research began on April 1, 2007. |
Research Project Status Report

Project: 06-407

Investigators: Jeffrey Savell, Kerri Harris, Alejandro Castillo

Organization: Texas A&M University

Title: Evaluation of Alternative Cooking and Cooling Procedures for Large, Intact Meat Products to Achieve Lethality and Stabilization Microbiological Performance Standards

Cost: $74,500

Timeline: One year

End Date: April 2008

Description: The goal of this project is to validate the safety of slower cooking and cooling times for large whole-muscle meat products which will meet FSIS lethality and stabilization microbiological performance standards. The specific objectives are: 1. to achieve lethality microbiological performance standards for slower heating times than those defined by Appendix A using alternative heating times and temperatures; and 2. to achieve stabilization microbiological performance standards for slower cooling times than those defined by Appendix B using alternative cooling times and temperatures.

The following items were included in the research protocol prior to finalizing the research agreement as requested by the AMIF Research Advisory Committee.

Selection of product from upper weight ranges: To ensure the experiment evaluates the worst case scenario, we will select product from the upper weight ranges. The hams purchased for this experiment will weigh between 23 and 27 lbs. The inside rounds purchased for the roast beef evaluation will be greater than 20 lbs.

Addition of sodium lactate and sodium diacetate: Due to the common usage of lactate and diacetate in the processing industry, a 3% solution of combined sodium lactate and sodium diacetate will be used.

Staphylococcus aureus inoculation: The protocol will be modified to inoculate the product with Staphylococcus aureus, and designed to determine the impact of processing times and temperature on the growth of Staphylococcus aureus and the potential for toxin formation.
Relative humidity verification: During thermal processing, internal and external product temperature, cooking time, and relative humidity will be documented for each treatment. Relative humidity will be maintained through the dry bulb and wet bulb settings in the smokehouse. The relative humidity will be documented by a chart recorder and can be verified throughout the cooking process with a portable relative humidity measuring device that can be placed in the smokehouse during various points of the cooking process.

Heat shock spores: Prior to evaluating alternative cooling procedures, the hams and roast beef will be inoculated with Clostridium perfringens spores that have undergone a heat shock process. This heat shock process is the same procedure used by Juneja et al. when evaluating the increased thermotolerance of Clostridium perfringens spores following sublethal heat shock. Before inoculation, the Clostridium perfringens spores will be heat shocked at 75ºC for 20 minutes. The product will be inoculated with the heat-shocked Clostridium perfringens spores and thermally processed. Following the thermal processing, products will be assigned randomly to one of the ten cooling treatments.

For the complete research description please contact Susan Backus at sbackus@meatami.com.

Status: AMIF has entered into a research agreement with Texas A&M University. Research will begin on May 1, 2007.
Research Project Funding Request

Project: 06-406

Investigators: Randy Wehling, Michael Zeece, Harshavardhan Thippareddi

Organization: University of Nebraska

Title: Evaluation and Analysis of Meat Products Contaminated by Low Levels of Ammonia

Cost: $67,350

Timeline: Two years

Description: The overall goal of this project is to develop a practical method for measuring ammonia contamination levels in meat products. The method will then be used to study the uptake of ammonia by meats, and the effectiveness of methods designed to decrease contamination levels. Specific objectives of the project are: 1) to develop methods for measuring ammonia contamination levels in meat products by potentiometry using ammonia-sensing electrodes, and to evaluate the reliability and detection limits for those methods; 2) to use the developed methods to monitor the rate of ammonia uptake by fresh and frozen meats exposed to ammonia gas at selected concentrations and times; and 3) to investigate selected techniques for lowering ammonia levels in contaminated meats, including air flushing, vacuum treatment, and rinsing with dilute organic acid solutions.

Experimental Plan: 1) The first step of the project will be to develop a satisfactory method for extracting the ammonia from contaminated meat samples. Samples will be extracted with an aqueous buffer/alcohol solution. Different combinations of buffer salts, buffer concentrations, and type of alcohol will be compared to maximize ammonia recovery, while minimizing the amount of protein in the extract. If necessary, surfactants will be incorporated into the solvent to disperse lipid, although it will be desirable to minimize lipid extraction to prevent fouling of the ASE. Extracts will be adjusted to alkaline pH (approximately pH 11) to insure that the ammonia is all in the volatile NH₃ state, with the exact pH optimized as part of the study. Both liquid membrane and solid state ammonia sensing electrodes will be investigated for quantitatively measuring the ammonia in the extracts. The electrodes will be calibrated using aqueous ammonium sulfate solutions as standards. The electrical potential (voltage) produced by the ASE is plotted against ammonia concentration to establish a standard curve.

Baseline levels of ammonia for uncontaminated beef will be compared using the ASE and the enzymatic (glutamate dehydrogenase) methods. If the ASE method yields results higher than the enzymatic procedure due to the presence of volatile organic amines, methods for removing these amines from the extract will be investigated. Extract clean-up procedures will focus on using solid phase...
extraction cartridges containing a reversed-phase adsorbent to remove the organic amines without removing the ammonia.

Once the extraction procedure has been optimized, beef (ground and intact muscle) samples will be prepared with different levels of ammonia contamination by exposure to ammonia contaminated air inside a glove bag. Samples will be exposed for different lengths of time to achieve pH changes ranging from 0 – 1.5 units. The ammonia concentrations of the samples will then be determined by both the ASE and enzymatic procedures, and used to evaluate the linearity, reproducibility and detection limits of the ASE procedure.

If the ASE procedure is found to be reliable, and volatile organic amines in the meat do not present a substantial problem, the use of a solid state electrode for in situ measurement of ammonia in beef samples will also be evaluated, and its results compared to both the enzymatic procedure and the ASE method using extraction. Elimination of the extraction step could further improve the speed and simplicity of the ASE method.

2) The method developed in part one will be used to monitor the uptake of ammonia by both frozen and fresh meat samples. Ground beef (90% lean) patties will be used for the study, due to their large surface area-to-weight ratio. A sample on a commercial polymer tray will be placed into a glove bag, into which air and ammonia will be introduced. Ammonia will be controlled at levels between 0 – 200 ppm, using a portable gas analyzer to monitor the concentrations. After exposing a sample to ammonia under selected conditions of temperature, concentration and time, the sample will be removed from the glove bag and immediately analyzed for ammonia using the method developed in part one. One set of experiments will be conducted using frozen patties at a temperature of -18 C, and a second set using fresh patties at a 3 C temperature. For each temperature, samples will be subjected to different combinations of ammonia concentration and exposure times. Times will be determined using results from part one. For each temperature, a response surface experiment will be designed to maximize the information available from a limited number of samples, and performed in duplicate. From these data, the relationships between time and exposure concentration for both fresh and frozen meat will be elucidated.

The experiments will then be repeated using ground beef samples on a tray with a polyethylene overwrap, and in a vacuum pack using a commercial barrier film. The results of these experiments will provide some initial data as to whether or not packaging materials affect the rate of uptake of ammonia by ground beef.

3) A ground beef patty will be exposed to ammonia contaminated air at 3 C in a glove bag to achieve a specific level of contamination, as determined in part 2. The bag will then be flushed with “clean” air at different flow rates and for different times to assess the effectiveness of air flushing in removing ammonia from the ground beef. Flushing times will be calculated as the number of complete exchanges of air in the glove bag. The samples will be removed from the glove bag and immediately analyzed using the ASE method developed in part 1. Again, a response surface design will be used to establish the relationship between time and flushing rate on ammonia reduction.
The effectiveness of using vacuum to decrease ammonia levels will be evaluated by placing ground beef patties with a specific level of ammonia contamination in a vacuum chamber at 3°C. The pressure inside the chamber will be reduced to <50 mm Hg with a vacuum pump. Samples will be removed from the chamber at 30 minute intervals, and analyzed for ammonia content using the ASE method. Treatment will continue until additional time in the chamber results in no further reduction in ammonia content. The experiment will be replicated three times.

The effectiveness of removing ammonia from contaminated meat by rinsing with dilute organic acid solutions will also be evaluated. While it is more likely that a rinsing technique would be applied to carcasses or carcass parts than to products such as ground beef, the ground beef patties will provide a convenient test product for these preliminary evaluations. Solutions of lactic and acetic acids, at either 1% or 2% concentration, will be used to spray rinse ground beef patties that have a specific level of ammonia contamination. The ammonia concentration of the ground beef will then be measured by the ASE method immediately after rinsing. The pH of the meat samples will also be measured before and after treatment. Rinse volumes will be calculated as a ratio of solution volume to surface area of the beef patty. A factorial design using two concentrations and three wash volumes for each acid will be used, with the experiment replicated three times. From the results obtained, the practicality of using acid rinsing to reduce levels of ammonia contamination can be assessed.

AMIF will recommend that researchers use Ion Chromatography instead of ASE as recommended by the AMIF Research Advisory Committee. AMIF will provide recommendations on the proposed ammonia removal techniques, air flushing, vacuum treatment, and rinsing with dilute organic acid solutions.

Status: AMIF is actively working with both industry and the University of Nebraska to ensure that the most accurate and applicable protocol will be used. Until and unless the protocol is finalized, AMIF will not initiate a research agreement.
Research Project Status Report

Project: 06-403

Investigators: M. Ellin Doyle, Ronald Weiss, Stacey Schultz-Cherry, Hon Ip, Michael Robach


Title: White Paper on Destruction of H5N1 Avian Influenza in Meat and Poultry Products

Cost: $10,000

Timeline: Five months

End Date: May 2007

Description: Scientific literature databases (PubMed and Food Science and Technology Abstracts), U.S. government publications from CDC and USDA, relevant government publications from other countries and WHO, and industry publications will be searched for information on destruction of avian influenza viruses, particularly the H5N1 strain. The search will target information on the distribution of the virus in different tissues and organs of infected animals, thermal and non-thermal methods for destruction of the virus in meat and poultry, other foods, and in vitro.

It is anticipated that currently available information specific to the destruction of H5N1 viruses in meat and poultry and other foods will be incomplete and these data gaps will be noted. Available information on effective methods for destruction of foodborne viruses, as related to their structure, will be included to indicate procedures that might be useful in controlling H5N1 viruses in meat and poultry products and should be further explored.

The specific goals of this research are to: (1) determine, from the scientific literature, the generally accepted time and temperature requirements for inactivation of the H5N1 virus in meat and poultry products; (2) review non-thermal methods for inactivation of the H5N1 virus and their effectiveness and practicality in meat and poultry matrices; and (3) determine current data gaps regarding inactivation of these viruses.

For the complete research description please contact Susan Backus at sbackus@meatami.com.

Status: AMIF has entered into a research agreement with University of Wisconsin. Research began on February 1, 2007. AMIF expects a preliminary report by early summer 2007.
Research Project Status Report

Project: 06-201

Investigators: Charles Carpenter, Jeffrey Broadbent

Organization: Utah State University

Title: Validation of Levulinic Acid for Topical Decontamination of Meat Surfaces

Cost: $63,263

Timeline: Two years

End Date: TBD

Description: Topical application of levulinic acid to carcasses, or to ready-to-eat meats, may provide the meat industry with an alternative intervention against pathogenic bacteria. The overall goal of the proposed research is to validate the extent to which topical application of levulinic acid results in surface decontamination of pathogenic bacteria, and (or) imparts residual protection against pathogen growth. There are four specific objectives in support of this overall goal:

Objective 1. Validate the extent of decontamination that results from topical application of levulinic acid to meat surfaces as compared to lactic and acetic acids. Studies for this objective will compare the extent to which topical application of various organic acids kill pathogenic bacteria previously inoculated onto meat surfaces. Meat samples will be inoculated with a significant load (10^7 CFU/cm^2) of pathogenic bacteria. After inoculation, samples will be dipped in water (control), 2% lactic acid, 2% acetic acid, or 2% levulinic acid at 130°C to replicate concentration and temperature values used for carcass spray in the industry. Some inoculated samples will not be washed, and will serve as a no-intervention control. Samples will be vacuum packaged and stored overnight at 4°C. Residual counts of pathogenic bacteria on each sample will be made, and extent of decontamination calculated as the log reduction in CFU in treated samples compared to the no-intervention control. The experiment will be replicated in four model systems reflecting significant areas of concern that have driven pathogen reduction efforts and federal regulations. These include: Escherichia coli O157:H7 inoculated onto pieces of beef carcass tissue; Salmonella spp. inoculated onto pork carcass tissue; Salmonella spp. inoculated onto chicken carcass tissue, and; Listeria monocytogenes inoculated onto slices of ready-to-eat turkey roll. Topical application of levulinic acid will be validated as an effective intervention for tissue decontamination if it reduces pathogen loads to a similar or greater extent than lactic and acetic acids.

Objective 2. Validate the extent of surface decontamination that results from topical application of levulinic acid at various concentrations and at temperatures greater than currently in use for lactic and acetic acid. Use of elevated
temperatures are a viable possibility with levulinic acid because of its much greater boiling point than lactic and acetic acids. These experiments will follow the protocols described for experiments under objective 1, and will only consider the model of *E. coli* O157:H7 inoculated onto pieces of beef. A complete factorial of wash treatments will be employed that includes dips in 0, 0.5, 1.0, and 2.0% levulinic acid at 130, 155, and 170°F. The results will validate options for topical application of levulinic acid that may have positive implications for food safety and cost effectiveness.

**Objective 3. Validate the residual protection against growth of pathogenic bacteria imparted by topical application of levulinic acid to meat surfaces as compared to lactic and acetic acids.** Studies for this objective will follow the growth of pathogenic bacteria inoculated onto meat previously dipped in various wash solutions. Meat samples will be dipped for 20 sec in water, 2% lactic acid, 2% acetic acid, or 2% levulinic acid at 130°C. After wash treatment, samples will be inoculated with $10^2$ CFU/cm$^2$ of pathogenic bacteria. Samples will be vacuum packaged and stored at low temperature. The model systems will be the same four described under objective 1. The samples of turkey roll inoculated with *L. monocytogenes* will be stored at 4°C, and pathogen counts determined at 0, 2, 4, 8, 12, and 16 weeks. All other samples will be stored at 8°C, and pathogen counts determined at 0, 2, 4, and 8 weeks. Topical application of levulinic acid will be validated to impart significant residual effect against pathogens if it inhibits growth of pathogenic bacteria to an extent similar or greater than lactic and acetic acids.

**Objective 4. Evaluate the organoleptic implications from topical application of levulinic acid as compared to lactic and acetic acids.** Studies for this objective will identify possible organoleptic consequences of using levulinic acid to treat beef trimmings and ready-to-eat meat. Beef trimmings will be sprayed for 20 sec with water, 2% lactic acid, 2% acetic acid, or 2% levulinic acid at 130°C. Trimmings will be ground and formed into patties. Color of patties will be instrumentally monitored during storage. Patties will be cooked and served to members of a consumer panel for evaluation of overall liking. In a companion study, slices of turkey roll will be sprayed with water, 2% lactic acid, 2% acetic acid, or 2% levulinic acid at 130°C. Once again, meat color (slices) will be instrumentally monitored during storage, and samples will be served to members of a consumer panel for evaluation of overall liking. Treatment with acids will be confirmed to have no negative sensory impact if acid-treated samples receive sensory scores that are similar to scores for the samples treated with water.

For the complete research description please contact Susan Backus at sbackus@meatami.com.

**Status:** AMIF is currently working with Utah State University to finalize a research protocol that will be most beneficial to the industry. Once that is complete, AMIF will enter into a contractual agreement with USU.
A great deal of the scientific knowledge describing the epidemiology of *Salmonella* in the pork chain is available as primary research; therefore the goal of this project is to synthesis/translate/combine the literature that describes the epidemiology of *Salmonella* in the pork chain using a systematic review. Current and future *Salmonella* public health risk assessment will benefit from the systematic review which will describe what literature is available about the epidemiology of *Salmonella* in the pork chain. Further this systematic review will identify areas where research is repetitive or lacking, and can be used as a resource allocation tool to “fill in the gaps” in risk assessment models. The process of systematic review reduces bias in the selection of research studies by the comprehensiveness and reproducibility of the search strategy and the transparent selection of articles included in review. Systematic reviews assess the methodological quality of the included studies (i.e. the rigor with which the study was designed, conducted and analyzed) and evaluate the overall strength of that body of evidence. For example, fields based studies better describe methods of introduction of *Salmonella* than simulated studies in laboratories, therefore information from well conducted field studies is given higher evidentiary value than the simulated studies. To accomplish the project goals a review advisory team will oversee a review team and the review will follow the steps of a systematic review 1) question design, 2) literature search 3) relevance screening 4) quality assessment 5) data extraction and 6) report synthesis.

The will be a review of the pork chain epidemiology focusing on primary research describing evidence for

1) Location of the pork chain of *Salmonella* introduction in the pre- and post harvest area;

2) Location of the pork chain of *Salmonella* amplification in the pre- and post harvest area;
3) A description of current available research reports and critical evaluation of the information contributed to understanding of pork chain Salmonella epidemiology. This summation will involve identification of study types currently being used to study the epidemiology of Salmonella in the pork chain and their evidentiary value; and

4) The final part of the report will provide a summary or the identified gaps and research priorities identified by the review.

For the complete research description please contact Susan Backus at sbackus@meatami.com.

Status: AMIF and NPB have entered into research agreements with Iowa State University. Research will begin on May 1, 2007.
Research Project Status Report

Project: 06-411

Investigators: Randall Phebus, Douglas Powell, Harshavardhan Thippareddi

Organizations: Kansas State University, University of Nebraska

Title: Beyond Intent: Assessment and Validation of On-package Handling and Cooking Instructions for Uncooked, Breaded Meat and Poultry Products to Promote Consumer Practices that Reduce Foodborne Illness Risks

Cost: $95,000

Timeline: Two years

End Date: January 2009

Description: The goals of this research are:

- To create a representative inventory of consumer handling and cooking recommendations on packages of heat treated not fully cooked, not shelf stable poultry products available at retail and for food service in the U.S. Specifically, uncooked, breaded, boneless poultry products that also may be stuffed or filled, charmarked, or artificially colored will be studied. These types of products have been implicated in a number of foodborne illness outbreaks.

- To undertake qualitative and quantitative research with consumers and food service employees to determine how various safe food handling statements are understood (or misunderstood), and the intention to act upon such understanding. Observation of product preparation by consumers and food service employees and in-depth interviews will be used to identify gaps between intention and actual behavior.

- To determine whether current labeling guidelines are effective in producing a safe end product if followed correctly under different preparation conditions using Salmonella-inoculated controlled cooking experiments.

There are two components to this research project. In the first component, a database of safe food handling statements on uncooked, breaded, boneless meat and poultry products will be compiled. A convenience sample of consumers will be asked to prepare a breaded, boneless poultry product using representative instructions created by the researchers, along with other foods to simulate the preparation of a full meal. Observation criteria will consist of incidences of cross-contamination, thawing techniques, label instruction reading, timing and thermometer usage. Following observations, in-depth interviews will be conducted which will provide researchers with insight to consumers’ views,
opinions and experiences. This observational study will be repeated with selected employees of food service facilities at Kansas State University.

In the second component, results from the observational studies will be used to design the laboratory research. Six products will be evaluated using multiple cooking protocols and product preparation combinations, including protocols defined by manufacturers on package labels and those that would likely be used by consumers or by food service personnel, as identified by the observational studies. Products will be thawed at 4°C (39.2°F) to allow inoculation with a multi-strain cocktail of Salmonella serovars. Products will be infused with the cocktail, held at 4°C (39.2°F) for 12 hours to allow diffusion of the inoculum throughout the product, after which they will be packaged, refrozen, held for seven days, and removed for cooking. A LabView multi-channel data acquisition system (National Instruments) will be used to monitor heating profiles across each product during cooking. Reductions in Salmonella populations will be determined based on uncooked inoculated control samples of each product.

For the complete research description please contact Susan Backus at sbackus@meatami.com.

**Status:** AMIF has entered into a research agreement with Kansas State University. Research began on February 1, 2007.
Controlling *Listeria monocytogenes* on Ready-to-Eat Meat and Poultry Products

I. **Innovative Pathogen Intervention Technologies**
   - Identification of bactericidal and/or bacteriostatic ingredients or treatments and the impact on FSIS regulations.
   - Identification of bactericidal and/or bacteriostatic ingredients or treatments that meet natural or organic requirements as defined by USDA.

II. **Operational Control and Monitoring of the Processing Environment**
   - Methods of preventing microbiological recontamination of sliced meats.
   - Validation of the expected impact of operational controls such as clean room technologies, facility and equipment cleaning procedures and environmental sampling plans.
   - Evaluation of real-time or near real-time *Listeria* sampling and testing technologies.
   - Development of improved quantitative methods for *L. monocytogenes* detection in foods and environmental samples.

III. **Information to Enhance Current and Future *Listeria* Risk Assessments**
   - Determination of quantitative levels of *L. monocytogenes* normally found on RTE meat and poultry when contaminated at time of processing.
   - Establishment of a protocol for validating acceptable log reduction for pasteurization of RTE meat and poultry.

Controlling *Escherichia coli* O157:H7 in Fresh Beef Products

I. **Pre-Harvest Research** (Pre-harvest is defined as the time period prior to cattle being placed on the trailer for transport to the slaughter facility.)
   - Develop greater understanding of the ecology/epidemiology of *E. coli* O157:H7.
   - Determination of the mechanism for intestinal colonization of *E. coli* O157:H7 and corresponding opportunities for control.

II. **Post-Harvest Research**
   - Verify lairage as a source of *E. coli* O157:H7 infections and, if verified, identify practical intervention technologies for use during lairage.
   - Investigation of novel intervention technologies for *E. coli* O157:H7.
   - Effect of the plant environment (e.g. air, machinery, employees) in the role of transmission of *E. coli* O157:H7.
   - Determine a novel method of reducing transfer of *E. coli* O157:H7 from hide to the carcass.
   - Determine the length of time that *E. coli* O157:H7 survives off the hide.
   - Develop easy to adopt hide treatment technology to reduce *E. coli* O157:H7 load on cattle presented for harvest.
   - Validation of existing intervention technologies for *E. coli* O157:H7.
III. Information to Enhance Current and Future *E. coli* O157:H7 Risk Assessments

- Research to address data needs identified in the FSIS Draft Risk Assessment for *E. coli* O157:H7.
- Develop data that may be used in future *E. coli* O157:H7 risk assessments.
- Determine the risk associated with non-O157 Shiga-toxin producing *E. coli* from beef products.

**Controlling Salmonella in Meat and Poultry Products**

I. Epidemiological Evaluation of *Salmonella* in Livestock Production and Processing

- Evaluate the effectiveness of production facility factors on prevalence of *Salmonella* in meat and poultry products.
- Evaluate the impact of on-farm management practices on *Salmonella* prevalence in livestock production and on meat and poultry products.

II. Innovative Pathogen Intervention Technologies

- Investigation and validation of novel intervention technologies for *Salmonella* in meat and poultry products.
- Determine the effectiveness of existing or new intervention technologies on multi-drug resistant *Salmonella* in ground beef.
- Identify the potential for *Salmonella* harbors within the post-harvest processing environment and interventions to reduce or eliminate the presence of *Salmonella* in the identified harbors.
- Identify likely sources of contamination, risk factors, and how to systematically intervene at the production facility, during transportation and lairage and the levels of *Salmonella* present on carcasses and meat products.

III. Information to Enhance Current and Future *Salmonella* Public Health Risk Assessments

- Conduct a Systematic Review of literature on pork chain epidemiology of *Salmonella*.
- Develop quantitative sampling and analytical methods for *Salmonella* on pork that will provide meaningful data for enhancing public health.
- Investigate the epidemiology of multi-drug resistant *Salmonella* within production and quantify the human health risks associated with these organisms.
- Determine the human health risk of *Salmonella* in livestock feeds.
Targeted Research

AMI member companies have identified very specific targeted research needs that will assist industry in solving unique technical challenges within meat and poultry facilities. These targeted projects have been specifically suggested by AMI member companies and some detail concerning project design has been provided. These projects will be considered for funding along with projects submitted for other research priority areas. In some cases, these targeted projects may overlap with the general research priority areas listed above. If you choose to submit a proposal to address one or more of these projects, please reference this intent in the pre-proposal.

Improve and augment epidemiological data on food attribution for listeriosis, both sporadic and outbreak cases.

The proposal should recognize the following assumptions:

- The Food and Drug Administration/Food Safety and Inspection Service *Listeria* Risk Assessment indicate ready-to-eat deli items are responsible for a majority of foodborne listeriosis cases in the U.S.

- USDA’s Food Safety and Inspection Service data indicate the prevalence of *Listeria monocytogenes* on RTE meat and poultry products has been declining from 2.54% contamination rate in 1998 to 0.55% in 2004, while the Centers for Disease Control and Prevention 2005 FoodNet data indicate listeriosis cases only declined from 5 cases/million in 1996 to 3 cases/million in 2005. If a majority of the listeriosis cases are indeed caused by contaminated deli meats a much more considerable decline of human listeriosis cases should have occurred over the last 9 years. Research is thus needed to understand the reason behind this apparent discrepancy between the risk assessment data and the FSIS and CDC data on food contamination with *L. monocytogenes* and human listeriosis cases in order to facilitate further targeted interventions to reduce human listeriosis cases.

Determine the rate of transfer of foodborne pathogens from food contact surfaces to meat and poultry products under commercial conditions.

The proposal should consider the following:


- Fresh and processed RTE meat and poultry products.

- Quantitative levels and serotypes of the pathogens.
Validation of on-package cooking instructions for uncooked, breaded, boneless poultry products that also may be stuffed or filled, charmarked, or artificially colored.

The proposal should address:

- Data needed to address concerns raised by FSIS (http://www.fsis.usda.gov/OPPDE/larc/Policies/Letter_to_Industry_on_Frozen_Uncooked_Poultry.pdf) describing the need to determine if consumers are aware of and complying with the cooking instructions.
- Data should also be provided on whether foodservice customers are aware of and complying with the cooking instructions.

Evaluation and analysis of meat products contaminated by low levels of ammonia.

The proposal should include the following:

- Evaluate various ammonia assays and determine those that can be used for in-plant rapid testing of potentially contaminated muscle food products.
- Identify the degree of product contamination that may occur during refrigerant leaks resulting in ambient air ammonia levels below 200 ppm.
- Determine ways (i.e., air flushing and packaging) to treat ammonia contaminated or exposed product so that it is acceptable for human consumption.

White paper on destruction of H5N1 avian influenza in meat and poultry products.

The white paper should include:

- Generally accepted consensus on the time and temperature requirements for inactivation of the H5N1 virus in meat and poultry products.
- Determination of current data gaps regarding this field of study.

Validation research to confirm safety of cooking and cooling procedures for large (>20 pounds) whole-muscle meat products (e.g., bone-in hams).

The proposal should address:

- Cooking and cooling processes for large whole-muscle meat products that achieve lethality and stabilization microbiological performance standards, but allow for slower heating and/or cooling times than those defined by Appendices A & B.
- Validation documentation from challenge studies, modeling or other means that industry can use to support the alternative processes.
Hot Topics
Clostridium difficile
Is *Clostridium difficile*-associated infection a potentially zoonotic and foodborne disease?

M. Rupnik

Institute of Public Health Maribor and University of Maribor, Faculty of Medicine, Maribor, Slovenia

**ABSTRACT**

*Clostridium difficile* has received much attention in recent years because of the increased incidence and severity of nosocomial disease caused by this organism, but *C. difficile*-associated disease has also been reported in the community, and *C. difficile* is an emerging pathogen in animals. Early typing comparisons did not identify animals as an important source for human infection, but recent reports have shown a marked overlap between isolates from calves and humans, including two of the predominant outbreak types, 027 and 017. *C. difficile* has also been found in retail meat samples, suggesting that food could be involved in the transmission of *C. difficile* from animals to humans.

**Keywords** Animals, *Clostridium difficile*, disease, foodborne disease, meat, typing

*Clin Microbiol Infect*

*Clostridium difficile* is a Gram-positive sporogenic anaerobic bacterium that can be a cause of intestinal disease, particularly following antibiotic treatment. It is usually considered to cause nosocomial infection, and most cases have been documented in the hospital environment [1]. Although the principal risk-factors are still prolonged hospitalisation, an age >65 years and antibiotic therapy, some recent changes in the epidemiology of *C. difficile*-associated disease have been observed. Overall, the incidence and severity of disease seem to be increasing [2], and reports of severe cases with a community onset are becoming more numerous. Furthermore, some of the community-acquired cases have occurred in a ‘low-risk’ population (i.e., young, without previous antibiotic therapy or previous hospitalisation) [3]. In addition, *C. difficile* has been recognised as an emerging animal pathogen [4].

There are several possible explanations for changes in the epidemiology of a pathogenic microorganism, including modification of the selection pressure in the existing environment (e.g., changes in the use of antibiotics in the hospital and non-hospital environments), the emergence of a novel variant or type of organism, and the introduction of a pathogen into/from a novel reservoir. The influence of antibiotic use on the incidence of *C. difficile* infections has been well-documented [5,6], and the way in which the epidemiology can change in response to a combination of antibiotic selection (fluoroquinolones) and the emergence of a new type is exemplified by recent outbreaks caused by type BI/NAP1/027 in the USA, Canada and some EU countries [1,6]. However, only part of the recent increase in mortality and morbidity caused by *C. difficile* infections can be accounted for by this new highly virulent type. Moreover, data published recently that compared human and animal isolates and revealed the presence of *C. difficile* in food now strongly suggest that animal reservoirs and transmission via foods are possible sources for community-associated infections.

Animals are an important source of human pathogenic microorganisms and can spread disease following direct or indirect contact, through environmental contamination or when used for food [7]. *C. difficile*-associated disease or asymptomatic carriage has been described in numerous animal species [4,8,9], but the *C. difficile* types in the human and the animal populations have not been compared in detail. The role of household pets as a potential reservoir for *C. difficile* infection...
was assessed as early as 1993, but no correlation was found between isolates from cats and dogs and isolates from humans in Australia [10], and it was concluded that these animals were not an important source of human infection at that time. As the human strains were isolated from the general population and not from the owners of the animals, no conclusion could be reached concerning possible household-related transmission between dogs/cats and humans.

Subsequent comparisons in Canada of small numbers of equine and dog isolates with human isolates have revealed that more than five ribotypes are found per host, and that different types are largely specific for each animal species. Only one of the ribotypes was detected in all three hosts; however, this ribotype accounted for 50% of all the isolates studied [11]. Colonisation of dogs with a highly virulent human strain and the potential role of dogs in transmission was exemplified by a hospital visitation dog that was colonised with epidemic type BI/NAP1/027 [12].

More recently, the same group of workers reported a surprisingly high degree of overlap in Canada between isolates from symptomatic and asymptomatic calves and recent human isolates [13]. All but one ribotype represented among isolates from calves had been recognised previously among isolates from humans. Indeed, two of the ribotypes identified have been associated with *C. difficile* outbreaks in humans, i.e., ribotype 017 (toxinotype VIII, A′B′CDT) and ribotype 027 (toxinotype III, A′B′CDT), while a third (078; toxinotype V) can be isolated readily from horses (personal unpublished results) and pigs (K. Keel, ClostPath 2006 Symposium, Nottingham, UK), and at least some of the ribotypes found (077, 014, M26, M31) were the same as those already found among isolates from dogs, calves and humans. Type M26 is identical in a number of molecular characteristics (toxinotype III, 18-bp deletion in *tcdC*, presence of binary toxin) to type 027, and was also resistant to levofloxacin and clindamycin, but the ribotyping and pulsed-field gel electrophoresis profiles were only 80% similar to 027 strains from humans. *C. difficile* could contaminate meat during processing, but another possibility is that spores are already present in the muscle tissue. This latter possibility has been described for other clostridial species in horses, but not, to date, for *C. difficile* [21]. Nevertheless, an increasing number of studies suggest that *C. difficile* strains can be transferred between humans and animals. Whether animals could serve as an important

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reservoir of C. difficile, with food as an important route of transmission, clearly needs further evaluation.

REFERENCES

Mycobacterium paratuberculosis Colloquium
Mycobacterium avium paratuberculosis: INFREQUENT HUMAN PATHOGEN OR PUBLIC HEALTH THREAT?

I. INTRODUCTION

*Mycobacterium avium* subspecies *paratuberculosis* (MAP) is a soil microorganism and the etiologic agent of Johne’s Disease (JD), a chronic and progressive enteric infection considered to be one of the most serious diseases affecting cattle [1] and other domestic and wild animals, including sheep, goats, elk, and primates. Clinical progression of the disease includes severe diarrhea and weight loss, and the affected animals eventually either die or are killed [2,3]. JD is prevalent in domestic animals worldwide; its economic impact in the U.S. alone is stunning, with an estimated loss of $1.5 billion every year [4]. One study estimated the prevalence of MAP in U.S. cattle to be 1.6%, with a significantly higher prevalence (2.9%) in the dairy cattle subset [5]. Studies in more localized herds of dairy cattle have produced even higher estimates; one estimated the prevalence of MAP in cattle in California to be 9.4% [6]. The problem is even more serious in other countries: a study in Denmark estimated the prevalence of MAP in the dairy cattle to be 47% [7,8].

Mounting evidence supports a role for MAP as an etiologic agent (although it may be one of several) of Crohn’s Disease (CD), a chronic relapsing inflammatory human disease of the gut. Symptoms include persistent diarrhea, cramping, abdominal pain, fever, rectal bleeding, loss of appetite, and weight loss. Some of this evidence includes:

- JD in cattle, which is caused by MAP infection, greatly resembles CD in humans in terms of the physical manifestations of the disease.
- Gross pathology and histopathology of JD and CD are similar [9].
- One study of the intestinal biopsies of CD patients found that MAP was present in 92% of all CD patients tested, as compared to MAP in only 26% of patients tested with noninflammatory bowel disease [10]
- Clinical trials of antibiotics to treat CD suggest some clinical improvement of patients [11, 12-15]. Although there are also a number of negative published studies, it should be noted that many of those studies attempted to treat CD with antibiotics that are ineffective *in vitro* against *M. avium* and MAP [11].
- MAP has been cultured from the breast milk of women with active CD [16].
- MAP has been detected in the cultured blood of patients with active CD [17].
- One case report describes a boy with MAP cultured from draining lymph nodes in his neck who developed typical CD 5 years later [18].
- MAP that was cultured from human beings caused typical JD when fed to baby goats.
- Epidemiologic evidence suggests that CD is increasing in prevalence, not only in the US, but also Japan [19] and Denmark [21].

It is possible that MAP not only causes JD in a wide number and variety of meat and dairy animals, but that at least some cases of CD could be zoonotic in origin, caused by direct or indirect transmission of the active MAP bacteria from animals (particularly cows) to human beings. While direct transfer has not yet been demonstrated, there is evidence that an unexpected percentage of the milk supply is contaminated with MAP from infected dairy cattle. A study on the detection of MAP in retail pasteurized whole milk in the UK showed that 7% of the
samples tested were positive for MAP by PCR [20], while another study in Switzerland found that nearly 20% of milk in tested bulk tanks contained MAP [22]. Another study provided evidence that MAP in naturally infected milk survived commercial HTST pasteurization when the bacteria were present in sufficient numbers [23]. If a solid link is established between MAP infection and even some CD, the public health implications of a contaminated retail milk supply are enormous. It will be vital to develop new methods of preventing MAP infection and transmission, and new diagnostics and treatments for MAP infection in both cattle and human beings.

II. NEED FOR AND TIMELINESS OF THE PROPOSED MEETING

The American Academy of Microbiology (AAM) plans to convene a colloquium entitled “Mycobacterium avium paratuberculosis: Infrequent Human Pathogen or Public Health Threat?” As discussed in the introduction, we believe that MAP is an existing underappreciated and emerging potential public health threat, and deserves a comprehensive examination of its role in animal and human disease and an evaluation of the events underlying MAP transmission from animals to humans.

The link between MAP and CD has been hotly debated for decades: because of the importance of this topic, the NIH convened a workshop to evaluate the evidence for a link, and the report of that workshop was published in 1999. The conclusion from the workshop was that there was insufficient data to either prove or disprove a cause and effect relationship between the bacterium and the disease. However, since that workshop, a number of interesting scientific findings have reopened the debate and rekindled interest in solving the mystery of the underlying cause of CD. Among the recent findings that may shed light on a link between MAP and any human infectious disease (including CD) are:

- Microbial etiologies (or putative etiologies) for chronic diseases long thought to be induced by other non-infectious causes (ulcers, cardiovascular plaques, etc.) [24]
- Ability to identify MAP in biopsies of human patients with CD and its relative absence in normal controls [25].
- Identification of MAP in biopsies from CD patients by FISH [26].
- Identification of a gene that influences susceptibility to development of CD in ~15% of CD patients, CARD15/NOD2, a gene that influences the ability of humans to mount an immune response to a component of the mycobacterial cell wall [27]
- Discovery and cure by antibiotics of a CD patient with both NOD2 gene and MAP infection.
- Results from four open-label clinical trials of antibiotics for the treatment of CD that demonstrated between 50-75% efficacy

Other meetings have been held on issues related to MAP, but no specific reports have emerged providing an objective analysis of the possible public health hazard represented by MAP. It is important to identify the gaps in our knowledge, and to focus new research to resolve these questions. We therefore believe that our report will fill a need for credible research recommendations for the future.

III. OBJECTIVES AND PRINCIPAL TOPICS TO BE COVERED

The AAM colloquium format is unique. The goals are to identify key disciplines and experts who, through working sessions over a 2 ½ day period, will synthesize the current status of the science and develop a report which can be used by government agencies, scientific and lay
communities, the public, and academics to answer critical questions of our time on particular topics.

The AAM will convene a colloquium—defined by Webster as “a discussion meeting”—of international scientific experts to develop the intellectual material for a written report. These experts will gather to consider each topic in a setting and format that encourages open and vigorous discussions. These deliberations will form the foundation for a report, published by the AAM, that will include a succinct description of the issues, graphical representations, where appropriate, and recommendations for future action.

The steering committee has composed a set of preliminary questions, intended to be the focus of the colloquium, and these are listed below. The bulk of participants’ time will be spent in small working groups addressing each of the questions that the steering committee has developed in advance. The colloquium is highly structured; however, there is sufficient time and flexibility for creative and spontaneous exploration of the issues. It will be made clear that these questions are the starting point for discussion; the groups will be free to explore issues as they arise in the course of deliberations.

**Environmental/zoonotic sources of MAP and control measures**

1. What is the prevalence of JD in cattle, both in the U.S. and globally?
2. What other domestic meat or dairy animals are infected with JD?
3. How is JD diagnosed currently, and are there new diagnostics in development? How accurate are these diagnostics?
4. What are the measures used in the control of JD, and how successful are they? Should animals who test positive for JD be killed and culled from the food chain?
5. What is known about the prevalence of MAP in the milk supply and its viability following pasteurization, both in the U.S. and globally? Does ultra pasteurization kill MAP any more effectively than regular pasteurization? What other strategies could be investigated to kill MAP in milk? What evidence is there that this is a source of MAP infection in humans?
6. Is there evidence for the transmission of MAP from cattle outside of the milk supply, or from other domestic animals?
7. What are the other zoonotic or environmental sources of MAP that could contribute to the spread of infection in humans? How are these controlled?
8. What is the prospect of finding a vaccine for MAP that could prevent transmission among animals, and possibly prevent the development of active CD in individual people who are genetically susceptible to MAP infection?
9. What is the evolutionary logic of host-parasite interactions as causes of chronic disease, as opposed to autoimmune mechanisms?

**Human MAP Infection**

1. What is the prevalence of MAP infection in humans?
2. What are the known/possible pathologies of MAP infection in humans, and what is the evidence for a direct link to MAP?
3. What are the methods of diagnosing MAP infection in humans, and how sensitive and specific are they?
4. What new laboratory techniques could be employed to detect MAP accurately in humans?

5. What are the methods of treatment of MAP infection in humans?

_Potential role for MAP in CD_

1. What methods are used to diagnose CD?
2. What treatments are used in CD, and how effective are they?
3. What are the side effects and risks of current approved treatments for CD?
4. What is known about genes related to susceptibility to CD?
5. What is the evidence for and against a role for MAP in CD?
   a. Are immune responses specific to MAP consistently reported in CD?
   b. Do tissue samples from CD patients contain MAP?
   c. What are the similarities and differences between the pathologies of CD and JD?
   d. Have antimycobacterial drugs been successful in the treatment of CD, and if so, is there a correlation to activity against _M. avium_ and/or MAP?
   e. What are the side effects and risks of antimycobacterial treatments for CD?
   f. Is there a correlation between incidence of CD and potential sources of MAP infection?

_Gap Analysis_

What additional information/research is necessary to further clarify the role of MAP as a human pathogen?

IV. LOCATION AND DATES OF COLLOQUIUM

The colloquium will be held June 15-17, 2007 in Salem, Massachusetts. This location has been selected because of its proximity to an international airport and reasonable hotel prices.

V. ORGANIZATION OF COLLOQUIUM, OUTCOMES, AND CONTRIBUTIONS

A group of 30-40 scientists with a broad range of expertise (ranging from basic science to clinical medicine, veterinary biology to human disease) will be invited to participate in this 2 ½-day colloquium. Attendance at the colloquium is by invitation only, and participants are selected to ensure the greatest scientific balance and diversity. The AAM, through funding supporters for this project, will provide travel support for colloquium participants, including airfare, ground transportation, on-site lodging and meals, and other expenses related to attendance.

The bulk of participants’ time will be spent in small working groups addressing each of the questions that the steering committee has developed in advance. The colloquium is highly structured; however, there is sufficient time and flexibility for creative and spontaneous exploration of the issues.

The general sessions will bring all colloquium participants back together to share the working group conclusions and recommendations and discuss any issues raised. Following the colloquium, a science writer (who will attend the colloquium), working closely with the steering committee chair, will develop a draft report for review by colloquium participants.

The agenda for the colloquium is as follows:
Day 1
Participants arrive; no scheduled events

Day 2
7:30-8:30 am  Group breakfast and information on scientific discussions
8:30-10:00 am  Welcome, introductions, and panel presentation of the issues
                Charge to participants
10:00 am-12:30 pm  Working groups
12:30-1:30 pm  Group luncheon
1:45-5:30 pm  Working Groups
6:30 pm  Group dinner and information scientific discussions

Day 3
7:30-8:30 am  Group breakfast and informal scientific discussions
8:30 am-12:30 pm  Working groups
12:30-1:30 pm  Group lunch and informal scientific discussions
1:45-5:30 pm  General session
6:30 pm  Group dinner and informal scientific discussions

Day 4
7:30-8:30 am  Group breakfast and informal scientific discussions
8:30 am-12:00 noon  General session
                Working group presentations
                Final remarks
12:00 noon  Adjourn

Following the colloquium, our science writer will draft a report, summarizing the deliberations of the working groups. The draft report will be sent to all colloquium participants for review, followed by peer-review by the AAM’s Board of Governors. The report will then be published and posted on the AAM’s web page. A specific outreach plan will be developed, including:

- Press release announcing the report will be sent to relevant scientific publications, such as Science, Nature, as well as to popular science publications, e.g., Science News, Scientific American, Discover, Popular Science, and New Scientist.
- Press release will be posted on EurekAlert, the web site for science journalists hosted by the American Association for the Advancement of Science (AAAS).
- The report will be posted to the web site for the American Society for Microbiology (ASM) and announced to the 43,000+ members of the Society through the “What’s New” section of the Society’s home page (http://www.asm.org). To date, there have been over 460,000 downloads of AAM colloquia reports.
An email announcement will be sent to all Fellows of the AAM, as well as members of relevant scientific divisions of the Society.

Copies of the report will be provided to the leadership of the ASM, colloquium supporters, colloquium participants, and member organizations of the International Union of Microbiological Societies.

Government agencies, industry, educators, and the scientific and lay communities have a strong need for objective, credible analyses, assessments, and recommendations on critical issues in microbiology. AAM colloquia are designed to evoke just such information. Our reports are viewed as unbiased statements of the issues and practical recommendations for the future.

**Contributions**

The predicted contributions to the enhancement and improvement of science are:

- Objective analysis of what is known about *M. avium paratuberculosis* as a public health problem; and
- Recommendations for future research to clarify the gaps.

**VI. STEERING COMMITTEE**

Members of the steering committee were selected as representative of the various stakeholders with interest in the issue of *M. avium paratuberculosis* as a potential public health threat, including infectious disease experts, practicing gastroenterologists, veterinary sciences and food safety experts, and the CD patient population.

*Chair:* Carol A. Nacy, Ph.D., Sequella, Inc, Rockville, MD

Marcel Behr, M.D., Ph.D., Division of Infectious Diseases and Medical Microbiology, McGill University, Montreal, QB, Canada

Charles Bernstein, M.D., Inflammatory Bowel Disease Clinical and Research Centre, University of Manitoba, Winnipeg, MB, Canada

Judith Eve Lipton, M.D., private practice, psychiatry; Distinguished Fellow of the American Psychiatric Association, and a patient with Crohn’s Disease, Redmond, WA

Mary E. Torrence D.V.M., Ph.D., DACVPM, National Program Leader, Food Safety, USDA, CSREES, Washington, DC

**VII. RECRUITMENT OF PARTICIPANTS AND SUPPORT**

Colloquia sponsored by the AAM differ significantly from traditional conferences in which formal presentations are made. Colloquia have no formal presentations, and honoraria are not provided.

All invited participants are assigned to working groups. The approximately 30-40 invited scientists represent the range of interdisciplinary approaches that can be applied to the issues. We have included a number of younger, but proven, scientists in our list of potential participants for their fresh perspective and to provide them the opportunity to deliberate with more senior scientists. It is critically important to have key international scientists to facilitate harmonization of goals and strategies from other parts of the globe.

The steering committee carefully selects each participant, giving all due consideration to including underrepresented minority and women scientists and scientists with disabilities. The
committee develops the intellectual approach to the issues, including drafting the questions to be deliberated at the colloquium. Then the committee determines the scientific disciplines that must be represented to maximize discussion and expertise, and from this list of disciplines the committee develops the final list of participants upon consultation with the Academy’s Committee on Diversity. The Academy’s Committee on Diversity’s mission is to increase the visibility and participation of underrepresented minority and women scientists in the Academy and its programs. One of the ways in which the committee achieves its mission is by direct participation in identifying scientists with appropriate expertise as potential colloquium participants.

**Potential Participants**
The steering committee has prepared a preliminary list of potential participants from both academics and industry, with interest and expertise in different areas including MAP microbiology, diagnosis, treatment, and human and animal health.

1) Dr. Peter Anderson, Statens Serum Institute, Denmark (mycobacterial cell-mediated immunodiagnosis)
2) Dr. John Bannantine, USDA Iowa (genomics of MAP)
3) Dr. Ed Boedecker, University of New Mexico (MAP in CD)
4) Dr. Tom Borody (formerly associated with early H. pilori studies, clinical trial of CD with antibiotics)
5) Dr. Sheldon Brown, Bronx VA (anti-mycobacterial drugs and MAP susceptibilities)
6) Dr. Wil Chamberlain, Texas Tech, (MAP in CD)
7) Dr. Rod Chiodini (MAP immunology)
8) Dr. Robert Clancy, Univ. of Newcastle, Australia (MAP expert)
9) Dr. Michael Collins, University of Wisconsin (Johne’s Disease)
10) Dr. Paul Coussens, Michigan (immunology of MAP)
11) Dr. Jay Ellingson, Marshfield Clinic (author: MAP in 2% of retail pasteurized milk from WI, MN, CA)
12) Dr. Brian Geisbrecht, University of Kansas (MAP proteins)
13) Dr. Leonid Heifets, National Jewish, Denver (Antimycobacterial agents)
14) Dr. John Herman-Taylor (2003 publication: 90% of Crohn’s Disease patients had MAP in biopsies)
15) Prof. Richard Hunt, McMaster University, Canada
16) Dr. Vivek Kapur, University of Minnesota (Network coordinator, Johne’s disease)
17) Dr. Mark Klassen, Canadian Beef Export group
18) Dr. Preston Linn (Alliance Manager/Business Development, BD Technologies)
19) Dr. Elizabeth Manning, University of Wisconsin (MAP pathogenesis)
20) Dr. Saleh Naser, University of Central Florida (MAP genome)
21) Dr. M. Netea, Nijmegen, the Netherlands (NOD2 gene as Mycobacteria sensor)
22) Dr. Norman Pace, Colorado (M. avium 16s identification)
23) Dr. Jim Rothel, Cellestis (Quantiferon testing)
24) Dr. David Russell, Cornell University (intracellular pathogens, including Mycobacteria)
25) Dr. Katherine Sacksteder, Sequella, Inc. (MAP diagnostics)
26) Dr. Balfour Sartor, University of North Carolina, (MAP in CD)
27) Dr. Fergus Shanahan, National University of Ireland (MAP microbiology)
28) Dr. Christine Schewe (validation of PCR-based detection of Mycobacteria)
29) Dr. Salman Siddiqi, Becton Dickenson, (Mycobacteria expert)
30) Dr. Christine Sizemore, National Institute of Allergy and Infectious Disease, NIH (Program Officer of TB, Leprosy and other Mycobacterial Diseases Branch)
31) Dr. Hong Tang, National Institutes of Health (organizer of past NIH/Crohn’s Disease workshop)
32) Dr. David Taylor, Salix Inc. (formerly of Johns Hopkins School of Public Health)
33) Dr. Michael Towns, Medical Director Becton Dickenson
34) Dr. Richard Whittington, Australia (MAP diagnostics, in sheep)
35) Dr. Lee Ann Jaykus, NC State (food microbiologist)
36) Dr. Garry Adams, Texas A & M University (Johnes coordinated agricultural project)
37) Dr. Yrjo Grohn, Cornell (Epidemiologist)

The AAM through its funding supporters, will provide travel supports for colloquium participants, including airfare, ground transportation, on-site lodging and meals, and other expenses related to attendance.