

national alliance for food safety

**NAFS**

a partnership for the science of safe food

# National Alliance for Food Safety Progress Report 2003

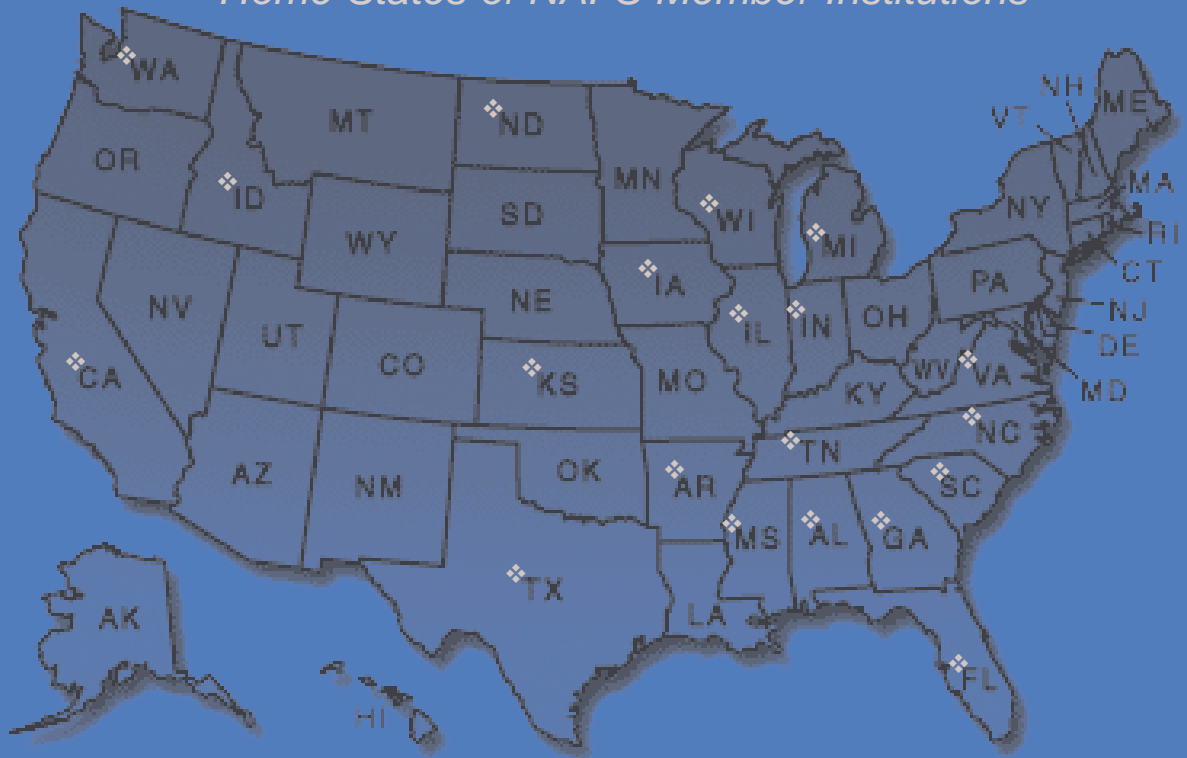


national alliance for food safety

**NAFS**

a partnership for the science of safe food

*Home States of NAFS Member Institutions*



*a partnership for the science of safe food*

# Contents

NAFS Member Institutions ...	1
NAFS Board of Directors ...	2
NAFS Operations Committee ...	2
Message from the Office of the Secretariat ...	3
Message from the Chair of the Operations Committee ...	4
NAFS Organizational Highlights ...	5
NAFS Research Accomplishments ...	8
Prevalence and Colonization of <i>E. coli</i> O157:H7 ...	8
Diagnostics, Risk Assessment and Control of <i>Listeria monocytogenes</i> ...	13
Microbial Controls in Plant Products ....	18
Future Plans for the NAFS and Funded NAFS Projects ...	23

## The Mission of the NAFS

The NAFS mission is to continually improve the safety and security of our food supply to ensure the public's health and to enhance our national and international food supply.

## Goals and Objectives of the NAFS

The goal of the NAFS is to conduct food safety and security projects assuring the highest applicability to food safety problems and being of the highest scientific merit.

The specific objectives of the NAFS are to:

- Meet the emerging food safety needs of industry in the areas of food production, processing, transportation, retail and food service;
- Address global issues in food safety related to the international marketing of U.S. agricultural products and enhance the safety of foods imported into the United States;
- Develop prevention and intervention strategies that will facilitate the continued production of healthy animals and safe plant-derived foodstuffs;
- Conduct research to enhance the safety of food products in the food service and retail environment, including market distribution;
- Communicate with the public regarding food safety research and implementation of technology for food products from production through the marketing chain to the consumer;
- Develop a framework for improving risk assessment and risk management information communication through a combination of resources including health, medical and epidemiological research programs;
- Increase our understanding of pathogens in the environment and food, including their prevalence, survival and adaptability under various conditions, and emergence of virulent strains;
- Addressing issues such as the role of food animals in the development of antibiotic-resistant human pathogens.

## NAFS Member Institutions

The National Alliance for Food Safety consists of a network of 20 universities and the U.S. Department of Agriculture Agricultural Research Service. NAFS provides the mechanism to enable participating universities and ARS to focus their collective scientific expertise and resources on all aspects of food safety. Frequent consultation with partners at government regulatory agencies is a major element of NAFS activities. Collaboration within the NAFS occurs among its partner members, government agencies, producer associations, private industry, and consumer organizations.

USDA Agricultural Research Service  
Auburn University  
Clemson University  
Iowa State University  
Kansas State University  
Michigan State University  
Mississippi State University  
North Carolina State University  
North Dakota State University  
Purdue University  
Texas A&M University  
University of Arkansas  
University of California-Davis  
University of Florida  
University of Georgia  
University of Idaho  
University of Illinois  
University of Tennessee  
Virginia Tech University  
University of Wisconsin  
Washington State University

Within the Alliance there are 12 Centers of Excellence that focus on a specific area of research regarding food safety. Each center has a Center Director and includes members with complementary areas of specialty from the NAFS universities. The centers provide a mechanism to promote inter-institutional and interdisciplinary problem-solving research. The Centers are:

<b>Beef Safety Center</b>	<b>Detection &amp; Typing Methods Center</b>
<b>Dairy Safety Center</b>	<b>Education &amp; Outreach Center</b>
<b>Plant Product Safety Center</b>	<b>Food Toxicology Center</b>
<b>Pork Safety Center</b>	<b>Microbial Physiology &amp; Ecology Center</b>
<b>Poultry Safety Center</b>	<b>Pathogen Control Center</b>
<b>Seafood/Aquaculture Safety Center</b>	<b>Risk Analysis &amp; Policy Center</b>

The NAFS acknowledges the vital support of the USDA Agricultural Research Service in the pursuit of its research and achievement of its objectives. Dr. Jim Lindsay (USDA-ARS), a member of the NAFS Operations Committee, administers ARS funding of NAFS research grants.

## **NAFS Board of Directors**

Gary R. Acuff  
Professor, Animal Science  
Texas A&M University

Douglas L. Archer  
Professor, Food Science and Human Nutrition  
University of Florida

Susan Barefoot  
Associate Dean, Food Safety and Nutrition  
Clemson University

Christine Bruhn  
Consumer Food Marketing Specialist  
University of California - Davis

Jerry Cherry  
Associate Dean for Research  
University of Georgia

Neville Clarke  
Program Coordinator  
Texas A&M University

James H. Denton, Chair of NAFS Operations Committee  
Center of Excellence for Poultry Science  
University of Arkansas

Ann Draughon  
Professor and Co-Director, Food Safety Center of  
Excellence  
University of Tennessee

Lee-Ann Jaykus  
Associate Professor, Department of Food Science  
North Carolina State University

Curtis Kastner  
Director, Food Science Institute  
Kansas State University

Ed Knipling  
USDA Agricultural Research Service

Jim Lindsay  
USDA Agricultural Research Service

Richard Linton  
Associate Professor, Assistant Director of Agricultural  
Research Programs  
Purdue University

Alan McCurdy  
Chair, Food Science and Human Nutrition  
Washington State University/University of Idaho

Lisa K. Nolan  
Associate Professor and Director of Great Plains Institute  
of Food Safety  
North Dakota State University

Don Reynolds  
Professor and Associate Dean for Research  
College of Veterinary Medicine  
Iowa State University

## *NAFS Board of Directors, continued...*

Robert W. Rogers  
Professor and Director, Food Science Institute  
Mississippi State University

Charles Scifres  
Deputy Director  
Texas Agricultural Experiment Station

Susan S. Sumner  
Professor and Head, Department of Food Science  
and Technology  
Virginia Tech University

Steve Taylor  
Head, Department of Food Science and Technology  
University of Nebraska

Laurian Unnevehr  
Professor, Agricultural and Consumer Economics  
University of Illinois

## **NAFS Operations Committee**

James H. Denton, Chair of NAFS Operations  
Committee  
Center of Excellence for Poultry Science  
University of Arkansas

Douglas L. Archer  
Professor, Food Science and Human Nutrition  
University of Florida

Susan Barefoot  
Associate Dean, Food Safety and Nutrition  
Clemson University

Neville Clarke  
Program Coordinator  
Texas Agricultural Experiment Station

James S. Dickson  
Professor, Department of Animal Science  
Iowa State University

Lee-Ann Jaykus  
Associate Professor, Department of Food Science  
North Carolina State University

Ed Knipling  
USDA Agricultural Research Service

Jim Lindsay  
USDA Agricultural Research Service

Richard Linton  
Associate Professor, Assistant Director of  
Agricultural Research Programs  
Purdue University

Charles Scifres  
Deputy Director  
Texas Agricultural Experiment Station

## Message from the NAFS Office of the Secretariat Charles J. Scifres Texas A&M University

The National Alliance for Food Safety was established November 1998 as a national network to focus research, education and service capacity of scientists from the land grant university and from the USDA Agricultural Research Service on immediate priority challenges to the nation's food system. The science capacity of this partnership includes 20 universities and literally hundreds of state and federal scientists. Since its inception, NAFS has invested more than \$3.51 million in priority food safety issues by supporting a broad set of research and service projects, each involving land grant university and ARS scientists.

Although initially emphasizing research, NAFS also engages in an array of service activities to assure its collective expertise is available as a rapid response mechanism for attacking issues of immediate concern. Thus, the investment in NAFS leverages resources for food safety research, provides a scientific resource for service activities and a powerful database for education activities — clearly the returns from the base investment have been magnified many times.

The NAFS is managed by an Operations Committee elected from the Board of Directors, which is composed of a member from each of the member universities. An annual meeting of the Board directs the course of NAFS, which is implemented in the interim by the Operations Committee. NAFS research is a partnership that reaches from the administration to the faculty who execute the projects. Dr. James Lindsay of the USDA-ARS provides administrative and management leadership for ARS funding of NAFS research and works as a member of the NAFS Operations Committee.

Funds were appropriated to the USDA Agricultural Research Service (ARS) for competitively awarded problem driven research conducted collaboratively by ARS and university scientists. Funds for the University-ARS partnership have been provided by appropriations to ARS at the level of \$1 million per year for the last three years for focused research on highest priority areas of relevance identified by industry-federal-university consultations. Early efforts were on *E. coli* and *Listeria* sp. Proposals have been competitively judged and 21 grants awarded to ARS-university collaborators to date. A symposium to report and discuss the results of these grants will be held in August 2003 in New Orleans.

Congress is requested to continue and increase the funding for this program to \$2 million per year for FY 04.

This first annual report illustrates the progress that is possible through an organized network capable of drawing on national scientific capacity. For additional information about NAFS, please contact the office of the NAFS Secretariat at 979-845-2855; email: [n-clarke@tamu.edu](mailto:n-clarke@tamu.edu); or via the Web: <http://nafs.tamu.edu>



**Dr. Charles Scifres**  
Deputy Director  
Texas Agricultural  
Experiment Station  
NAFS Secretariat

## Message from the Chair, NAFS Operations Committee, Board of Directors



Dr. James Denton  
Chair, NAFS  
Operations Committee  
Center of Excellence  
for Poultry Science  
University of Arkansas

The annual report of the National Alliance for Food Safety (NAFS) provides highlights of the accomplishments of the organization from two levels: service activities and research highlights. The accomplishments from the NAFS Operations Committee summarize three separate items in which the NAFS provided service to the USDA-Food Safety and Inspection Service Third Party Review of HIMP (HACCP Inspection Models Project), the Institute of Food Technologists International Food Safety and Quality Conference and the USDA-FSIS *Listeria* Summit Comments on Research Needs from the Perspective of Academia.

These service activities were made possible by the rapid response capability of the NAFS and the availability of the widely respected scientific faculty in the NAFS. The Third Party Review of HIMP provided the scientific assessment of the project which will allow the project to move forward as the next generation of inspection methods is adopted by USDA-FSIS. It will serve as a guide for the development of food safety policy which will assure that the best available systems are implemented. The comments provided on behalf of NAFS regarding research priorities for addressing *Listeria monocytogenes* will serve as part of the useful guide in addressing this very difficult and challenging food safety pathogen in developing sound food safety policy and effective food safety assurance systems. The engagement of the NAFS in co-sponsoring the IFT International Food Safety and Quality Conference is a strong statement to the effectiveness of NAFS in addressing complex scientific issues and providing the necessary expertise to assist in bringing the international scientific community to the forum for meeting the emerging challenges of the international food marketplace.

The research highlights section summarizes the activities in addressing the primary pathogens of concern, *E. coli* O157:H7 and *Listeria monocytogenes*. The control of *E. coli* O157:H7 in ground beef products is an issue of extreme importance in raw products that requires improved understanding of the microbial ecology in the live animal production environment, an area in which some progress has been reported by NAFS investigators. The control of *Listeria monocytogenes* in ready-to-eat meat products as well in plant products is also an issue of major concern in which some progress has been reported by NAFS scientists. Another section addresses the control of multiple pathogens in plant products. The control of these human pathogens takes on added significance in the context of international marketing of products imported into the U.S. Clearly the efforts of the NAFS are addressing issues of national importance in both research and service-related activities.

## National Alliance for Food Safety Invited to Provide Stakeholder Perspectives on Research Priorities for *Listeria monocytogenes* at USDA-FSIS *Listeria* Summit

### Major Issue

The USDA-FSIS hosted a one day “*Listeria* Summit” to obtain comments from experts in government, academia, industry and consumer organizations in providing current thinking regarding research priorities in risk assessment, risk management, risk communication and stakeholder perspectives for each group of experts regarding *Listeria monocytogenes*. The National Alliance for Food Safety was invited to provide stakeholder perspective from the standpoint of academia on promoting research on *Listeria monocytogenes*. The comments were prepared and provided by Drs. Sophia Kathariou and Lee-Ann Jaykus from North Carolina State University with input from ILSI, CDC, and FDA.

### Relevance of problem

The control of *Listeria monocytogenes* (*L.m.*) in ready to eat foods is an issue of considerable concern. This relatively new pathogen was first associated with milk and dairy products and has since been associated with other refrigerated foods. It has also been demonstrated to be a persistent organism in the environment of processing plants because of the extensive use of refrigeration as a control mechanism for many other pathogens. The organism flourishes in refrigerated conditions and therefore has become a significant hazard in several ready-to-eat food items including processed fully cooked meats and fresh fruits and vegetables. Because of this combination of circumstances in the food marketing system, control of this human pathogen has become an issue of great concern to the food industry, the regulatory community and the consuming public.

### Significant achievement

The NAFS partnership with the ARS has resulted in the initiation of a number of studies on *L. monocytogenes*, including those relating to virulence mechanisms, detection and control strategies, as well as studies to that complement risk assessment (exposure assessment and hazard characterization). Further investigation into the sources of contamination as well as the environment which permits growth of the organism is being conducted. While there is still much to learn on *Listeria*'s physiology, ecology and evolution it is imperative that the strategy taken to address these complex issues be done cooperatively. Only joint efforts which integrate academia, the food processing industry, the public health sector and other regulatory authorities will allow a better understanding of how to reduce the threat of *Listeria* as a food safety concern.

### Expected accomplishment

The NAFS partnership with the USDA-FSIS in addressing the priorities for the research agenda regarding control of *Listeria monocytogenes* should result in the effective utilization of precious resources for enhancing the understanding of this complex human pathogen. Conducting these discussions in a forum of this nature is essential to fostering greater understanding of the fundamental issues regarding effective food safety strategies.

### Partnership

The partnership represented by NAFS and ARS with the USDA-FSIS provides an excellent example of how truly effective university-ARS alliances can be in meeting the needs of an ever-evolving and more global food industry for which challenges are constantly emerging.

### Relevant presentation/publication

The presentation of these discussions during the USDA-FSIS *Listeria* Summit in Washington, D.C. makes them part of the agency record for use in setting research priorities for the control of *Listeria monocytogenes*.

## **National Alliance for Food Safety Task Force Third-party review of the HACCP-based Inspection Models Project (HIMP)**

### **Major issue**

The HACCP-based Inspection Models Project (HIMP) has come under criticism from several groups, including consumer interest organizations as well as members of the scientific community. The foci of these criticisms were made public during the meeting of National Advisory Committee for Meat and Poultry Inspection (NACMPI) in May 2002. The recommendation of the NACMPI was for the USDA-FSIS to engage a third party to review the experimental design, data collection and analysis and conclusions drawn from the original HIMP conducted by RTI, Inc. The National Alliance for Food Safety (NAFS) assembled a team of nationally and internationally recognized experts in poultry health, food microbiology, poultry processing and bio-statistics from the faculty membership of the NAFS. The team, chaired by Dr. Billy Hargis, Professor of Poultry Science at the University of Arkansas, included Dr. Mike Johnson, Professor of Food Science at the University of Arkansas, Dr. Pat Curtis, Professor of Poultry Science at Auburn University and Dr. John Williams, Professor of Bio-statistics at Texas A&M University.

### **Relevance of the problem**

The HIMP is a major endeavor undertaken by the USDA-FSIS in response to the 1996 Pathogen Reduction Act and HACCP Implementation legislation, an activity required in moving to a more science-based inspection system for assuring the safety of the U.S. meat and poultry supply for American consumers. The HIMP will form the basis for the next generation of meat and poultry inspection systems employed by regulatory personnel while working in concert with the meat and poultry industries to modernize the inspection system.

### **Significant achievement**

The NAFS Task Force completed the Third Party Review of HIMP and presented the results of the review during the November 2002 meeting of the National Advisory Committee for Meat and Poultry Inspection in Washington, D.C. The Third Party Review was published as part of the deliberations of the NACMPI.

### **Expected accomplishment**

The review recommendations will be used in guiding policy decisions of the FSIS regarding meat and poultry inspection systems as the expected continuous improvements in the system occur during the full implementation of advances resulting from HIMP.

### **Partnership**

The partnership represented by NAFS and ARS provided the mechanism required to assemble the nationally and internationally recognized team of scientific experts on very short notice, with the resulting rapid response to the critical need for evaluation of the HIMP prior to moving ahead with modernization of the meat and poultry inspection system. Without this mechanism it is unlikely that the task force could have been assembled in the timely fashion required to respond to these important concerns.

### **Relevant presentation/publication**

The presentation of the results of the Third Party Review of HIMP was conducted in the NACMPI meeting in Washington, D.C. in November 2002 and subsequently in another presentation in December 2002. In addition the written report of the Third Party Review of HIMP has become a part of the permanent record of the NACMPI deliberations.

## **National Alliance for Food Safety to Cosponsor the Institute of Food Technologists International Food Safety and Quality Conference in Orlando, Fla., November 5-7, 2003**

### **Major Issue**

The Institute of Food Technologists (IFT) International Food Safety and Quality Conference is structured to provide national and international leaders in food safety the forum to discuss significant issues facing the food industry. It also provides the opportunity to learn about cutting edge research/education advances from leading scientists engaged in food safety research and education. Addressing food safety issues in a comprehensive farm-to-table approach offers a great opportunity for improving the safety of the food supply. Five plenary interactive sessions have been developed that engage consumer food safety information, pre-harvest controls, and technologies for microbial control. New and innovative methods of detection and risk assessment are planned to address all parts of the food system in contemporary issues. Individual speakers from the National Alliance for Food Safety (NAFS) are featured in each of the sessions in the conference as well as the exhibits and poster sessions. NAFS scientists are also serving as the session co-chairs for each plenary session.

### **Relevance of problem**

The changing situation of the food industry results in a more global marketplace with many products being imported into the U.S. from a multitude of foreign countries and many of the companies in the U.S. now export food products to the entire globe. Addressing these complex issues in a format where scientists and industry leaders can engage in discussions of common concern and with the exchange of current information from cutting edge research for addressing specific challenges to the food industry is unique to this conference. Jointly addressing these challenges and finding common solutions to common problems serves the entire consuming public in a fashion which gives the industry the best opportunity to improve the safety and quality of the food supply.

### **Significant achievement**

The NAFS partnership with the United States Department of Agriculture Agricultural Research Service (USDA-ARS) has facilitated development of leadership groups of scientists in several disciplines by combining the individual strengths of the faculty from individual universities in a stronger and more responsive research and education organization. By capturing these strengths, the NAFS can provide unique dimensions to the IFT-International Food Safety and Quality Conference that are required to make advances required by today's society.

### **Expected accomplishment**

The NAFS partnership with the IFT in conducting this International Food Safety and Quality Conference should result in the establishment of the conference as the premier conference of this type. Providing a forum of this stature is essential to fostering greater international understanding of the fundamental issues regarding food safety and quality.

### **Partnership**

The partnership represented by NAFS and USDA-ARS with the IFT provides an excellent example of how truly effective university-government alliances can meet the needs of an evolving and more global food industry.

### **Relevant presentation/publication**

The relevant publications and presentations are as yet to be documented, although there are significant activities anticipated at the IFT-International Food Safety and Quality Conference in Orlando, Fla., Nov. 5-7, 2003.

## Factors contributing to the presence of *Escherichia coli* O157:H7 in feedlots and feedlot cattle

Elsa A. Murano and Gary R. Acuff, Texas A&M University; James S. Dickson, Iowa State University; Merle Pierson, Virginia Tech University; L. Wayne Green and John Sweeten, Texas A&M University Center at Amarillo, TX; Nolan Clark and C. William Purdy, USDA-ARS laboratory at Bushland, TX; Irene Wesley, USDA-ARS National Animal Disease Center at Ames, IA

### Major issue

Control of *E. coli* O157:H7 in beef products will rely heavily on enhanced understanding of the microbial ecology of the organism in the environment where beef cattle are produced. Factors which may influence the survival and growth of *E. coli* O157:H7 include the feedlot environment, intrinsic factors (ambient temperature, pond water temperature and electrical conductivity, liquid level of ponds) and weather-related factors. Another important element is correlation of environmental *E. coli* O157:H7 to presence of the pathogen in animals sold for slaughter.

### Relevance of problem

Recent studies have shown that the prevalence of *E. coli* O157:H7 in cattle may be higher than originally thought, mainly due to the use of new methods based on immunomagnetic bead capture. Elder et al. reported that 72% of the lots sampled had at least one positive fecal sample. The overall prevalence of *E. coli* O157:H7 in feces and on hides was found to be 28% and 11%, respectively, which are much higher figures than previously assumed. We have investigated the factors during cattle production that may contribute to the presence of *E. coli* O157:H7 in feedlots by monitoring the environment at the feedlot using the immunomagnetic bead method, followed by confirmation of positive isolates by PCR. In so doing, we have attempted to determine whether certain factors related to the feedlot environment impact the contamination of cattle leaving the feedlot.

### Significant achievements

A total of 1,125 environmental samples were collected once per month (July 2001 to March 2002) from five feedlots in the Texas Panhandle and examined for the presence of *E. coli* O157. Available samples collected from the feedlots included 229 chute samples, 399 water samples from feedlot lagoons, 399 samples of muck from feedlot lagoons, 55 drainage samples, 23 overflow pond water samples, and 20 overflow pond muck samples. Approximately 4% (47/1125) of the total samples collected were positive for *E. coli* O157. In addition, at least one sample from each feedlot was positive 42% of the time (19 of 45 sample occasions). Over the 9-month sampling period, January was the only month in which *E. coli* O157 was not detected from any of environmental sample sources, and positive environmental samples were most frequently detected in November and March (9 and 10% respectively). The number of individual positive samples for chutes, water, muck, drainage, and overflow pond of the five feedlots were 14 of 229 (6%), 9 of 399 (2%), 22 of 399 (6%), 1 of 55 (2%), and 1 of 43 (2%), respectively. More positive samples were found from feedlot 5 (10/143) than any other feedlot examined during this study and the greatest variation in positive samples between feedlots (0-34%) occurred during the month of March. Forty seven of the isolates originated from environmental sources, and 56 isolates were from the hide. The majority (64%) of isolates from feedlots were identified as H7, and 36% were identified as NM (O157:nonmotile).

During the 9-month period, several environmental factors were monitored in order to determine the possibility of a correlation with the presence of *E. coli* O157:H7 in the feedlots. None of these factors appeared to be associated with prevalence of *E. coli* O157 in environmental samples. It is possible, however, that the inability to isolate the bacteria in January may be associated with the occurrence of extremely cold weather conditions.

Sponge samples obtained from the hide of stunned adult cattle at a slaughterhouse facility indicated 56% (56 of 100) of the hide samples were positive for *E. coli* O157. Fourteen percent (7 of 50) of hides were positive for the bacteria on the first sample day and 98% (49 of 50) were positive from the second day.

**Expected accomplishments**

During the course of this study, *E. coli* O157 was isolated from all environmental sources, with peak prevalence during February and March. Cattle hide surfaces were found to be a significant and common source of the bacteria. All strains of *E. coli* O157 isolated in this study were closely related with regard to rep-PCR DNA fingerprinting; however, the majority of the hide isolates grouped together within one cluster. This finding may indicate the possible persistence of this specific strain on cattle hides. In addition, recovery of this strain from hides and from the feedlot environment on multiple visits may indicate that the maintenance, transmission and persistence of this strain is enhanced by the cattle production environment. Data collected in this study may be of assistance in developing strategies for adjusting management practices at feedlots in an effort to minimize the potential for contamination of cattle with *E. coli* O157:H7.



## Role of long polar fimbriae in intestinal colonization by *E. coli* O157:H7

N. Cornick, H.W. Moon, J.B. Kaper, T.A. Casey, E.A. Nystrom

### Major issue

*E. coli* O157:H7 is an important zoonotic pathogen that is usually transmitted to humans by the ingestion of contaminated food or water. The natural reservoir of *E. coli* O157:H7 is thought to be ruminant animals although the organism has been isolated from a variety of both domestic and wild animals. In the United States approximately 2-28% of healthy adult cattle shed the organism. *E. coli* O157:H7 produce several known virulence attributes that are involved in pathogenesis. One of these factors, intimin, is required for the formation of attaching and effacing (AE) lesions in both neonatal calves and pigs. Intimin is prerequisite for the *E. coli* O157:H7 to be pathogenic in these animal models. Attaching and effacing lesions have not been detected in mature cattle but occasional lesions have been found in experimentally inoculated, clinically healthy, weaned calves. In addition, we have shown that intimin facilitates the colonization of *E. coli* O157:H7 in mature, asymptomatic ruminants.

### Relevance of problem

The long-term goal of both ARS and ISU co-investigators has been the development of a vaccine to eliminate the carrier-shedder state of *E. coli* O157:H7 in cattle. Currently work is in progress and has focused on intimin (an outer membrane protein required for attaching/effacing activity of *E. coli* O157:H7) as a potential vaccine antigen. It has long been suspected that in addition to intimin, *E. coli* O157:H7 has fimbriae required for interaction with the intestinal surface. One of the Co-PI's on this project (Kaper) has identified an operon of *E. coli* O157:H7 that has significant homology with the long polar fimbriae (LPF) of *Salmonella typhimurium*. LPF contributes to the attachment and colonization of Peyer's patches in the small intestine of mice. Isogenic strains of *S. typhimurium* that have a mutation in *lpf* gene are attenuated compared to the wild type and the LD50 is increased approximately 5-fold in orally inoculated mice. The existence of a homolog to LPF in *E. coli* O157:H7, but not in *E. coli* K-12, suggests that LPF may play a role in colonization of the intestinal tract by *E. coli* O157:H7 as well.

### Significant achievement

Our results suggest that LPF may contribute to the initial binding and colonization of *E. coli* O157:H7 in ruminants, but that other factors must compensate for the lack of *lpf* since all of the sheep inoculated with the *lpf* mutants continued to shed those strains for at 2 months after inoculation.

### Expected accomplishment

The objective of this project is to determine if LPF contributed to persistent colonization in the sheep model of the ruminant carrier-shedder state for *E. coli* O157:H7. The approach will be to utilize isogenic mutants of a strain of *E. coli* O157:H7 with and without the *lpf* gene.



## Epidemiological associations of *E. coli* O:157 from produce, environmental samples, and animals

Ann Draughon, Ph.D., The University of Tennessee, Food Safety Center of Excellence, David Golden, Ph.D., Alan Mathew, Ph.D., Steven Oliver, Ph.D., Dale Hancock, Ph.D., DVM, Washington State University, Field Investigation Unit, Robert Mandrell, Ph.D., USDA-ARS, WRRRC-Albany, CA.

### Major issue

Changes in agricultural practices regarding production of produce and animal husbandry have had significant impacts on the occurrence and distribution of bacteria causing foodborne diseases geographically and across the animal and plant kingdoms. However, quantifying and evaluation of the impact of those practices has been difficult because of a lack of scientific data. The challenge was further complicated because optimal methods to isolate and identify the disease-causing foodborne bacteria in the farm environment had not been investigated and validated. The goal of this project was to develop and validate the methods needed for isolation of foodborne pathogens from animals and to then determine the prevalence of such pathogens in food animals and their environment so that risk analysis could be performed and risk management used to reduce the risk of foodborne diseases from animal products.

A second goal of this project was to develop a highly talented research team from cross-disciplinary fields and across institutions (The University of Tennessee, Washington State University and USDA) which could tackle complex food safety research problems. The problems associated with the safety of our food supply are not simple to solve. They are complex since food safety issues begin with tiny bacteria having complex genetic differences which may rapidly change beginning at the farm and ending with the consumer. The team which we put together included a veterinarian, an animal scientist, an environmental scientist, a food microbiologist, a molecular biologist, a biochemist and a risk assessment expert. This was the first experience of these scientists in working in a true collaboration between them and between academia and USDA-ARS. Over the last three years, this project team had continued to expand, communicate and collaborative on research problems in a highly effective manner which has led to numerous breakthroughs in food safety research.

### Relevance of problem

Contamination of fruits and vegetables and meat products with bacteria which cause foodborne illness has become an increasing problem over the last 20 years. Animal agriculture and plant agriculture are inevitably linked to one another because of the nature of our planet ecosystem. Animals produce waste which becomes a source of nitrogen which is needed to make plants grow. In turn animals eat plants and continue the cycle. Unfortunately, billions of bacteria live in each animal which is also released into the environment with their waste. As animal populations on the planet continue to increase, this becomes an increasing problem because of the genetic diversity of microorganisms - some of which cause human illness. Also, there is a need for centralization of animal and plant production to make it economical which concentrates the contamination problem in certain locations. *E. coli* O157:H7 ("hamburger bacterium") is a particularly dangerous foodborne microorganism since it can rapidly cause death and/or kidney failure in young children.

We do not currently have good epidemiological or risk assessment models to predict the impact of environmental or animal contamination on the occurrence of disease-causing bacteria in the food system. Therefore, studies such as our project are needed to provide critical information on predicting risk and protecting the food supply.

**Significant achievement**

Occurrence of *E. coli* O157:H7 was found to occur with a high seasonal preference for summer and fall in cattle. Little or no *E. coli* O157:H7 was isolated in winter and spring. *E. coli* O157:H7 was also isolated from chickens but not swine in our study from 16 farms nationwide. The major environmental sources on the farms found to be contaminated with *E. coli* O157:H7 were soil, insects, watering troughs and wild birds.

**Expected accomplishment**

Year 1: Methodology for isolation of *E. coli* O157:H7 was developed and validated for isolation of this organism from beef cows, dairy cows, swine and poultry. Methods were also developed for isolation of *E. coli* O157:H7 from insects, farm water, biosolids ponds, wild birds, rodents, mice, air and feed supplies.

We will develop a risk assessment model using our database with a series of computer programs by ESRI using GIS (geographical information system) which places scientific data in colored maps which permit easy visualization of areas with very high, very low or no contamination with *E. coli* O157:H7 pathogen. This will permit more effect risk management decisions.

**Partnership**

Our NAFS seed grant lead to a proposal by our group which was successful in establishment of a \$5 million dollar (5-year, \$1 M /year) Food Safety Center of Excellence in Tennessee. This Center was funded entirely by the State of Tennessee using a competitive process. Without the collaborations established during the NAFS proposal process, the Center would not have been funded. In addition, in 2002, the research team organized for this proposal was successful in obtaining a USDA-IFAFS funded project (\$599,000) on the epidemiology of four major foodborne pathogens in food animals. The FDA Center for Veterinary Medicine in 2001 provided \$450,000 to assist in developing the methodology initiated in this proposal. Our most recent collaborators to join the team is the Oak Ridge National Laboratory Risk Assessment Team which provides expertise on programming of databases and GIS expertise.



## Predictive risk assessment for ingestion of *Listeria monocytogenes*

Charles J. Czuprynski (Univ. Wisconsin), John B. Luchansky (USDA, Wyndmoor, PA)

### Major issue

Listeriosis is an important foodborne disease in the United States. It is estimated that there are approximately 2,500 cases per year and up to 500 deaths related to listeriosis. Our understanding of factors that influence the pathogenesis of listeriosis is limited. Major outbreaks of listeriosis occurred in 2002 related to contamination of turkey meat with *Listeria monocytogenes*. This project will help to elucidate how growth of *L. monocytogenes* on ready-to-eat (RTE) meat products might influence the pathogenesis of listeriosis.

### Relevance of problem

Listeriosis is a significant food problem in the United States (2500 cases per year and up to 500 deaths). Although the number of cases is not great in comparison to some other foodborne disease pathogens (i.e. *Campylobacter*, *Salmonella*), it causes more deaths than any other foodborne bacterial pathogen. Contaminated food products, of both animal and vegetable origin, can support the growth of *Listeria monocytogenes*. What makes this organism particularly dangerous is its ability to multiply, at both refrigeration and ambient temperatures, in common food products. Furthermore, it is an intracellular pathogen whose site of residence in the human body during the early stages of infection is not known. Understanding the pathogenesis of this complex agent is, therefore, important if we are to reduce the risks associated with food contaminated with *Listeria monocytogenes*.

### Significant achievement

Using outbred mice, our laboratory demonstrated that, serotype 4b strains of *L. monocytogenes* are more virulent in intragastrically inoculated mice than the other strains examined. Although only a few strains were used, this finding is consistent with the association of serotype 4b strains with large outbreaks of listeriosis, and with other published reports using animal models.

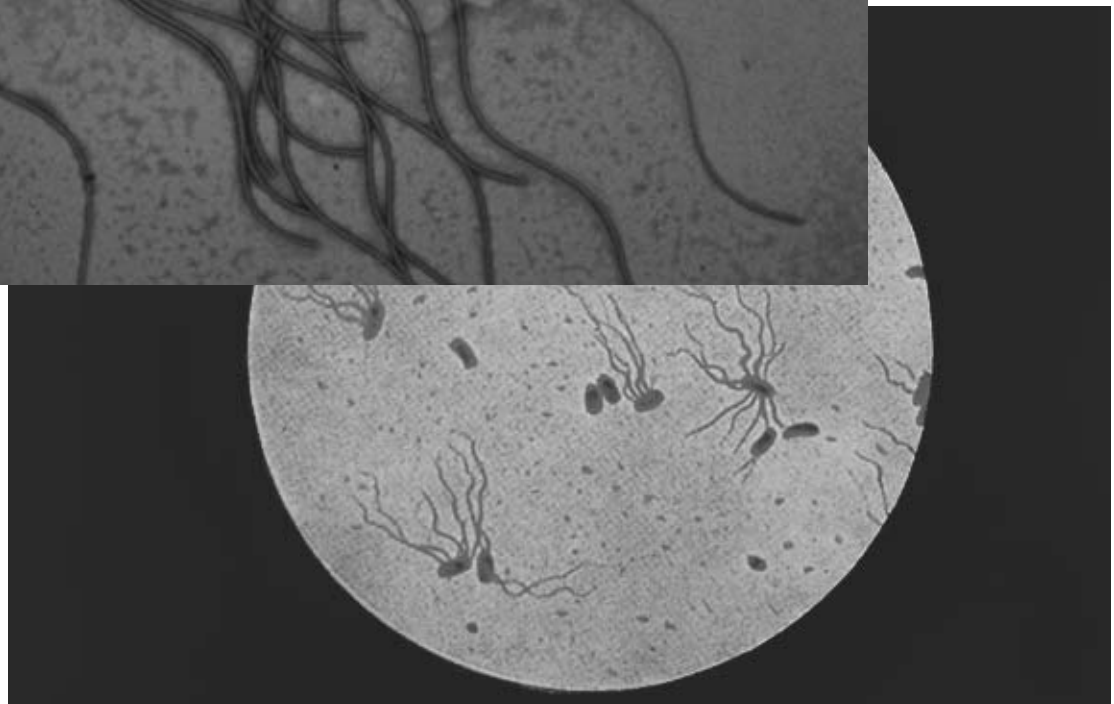
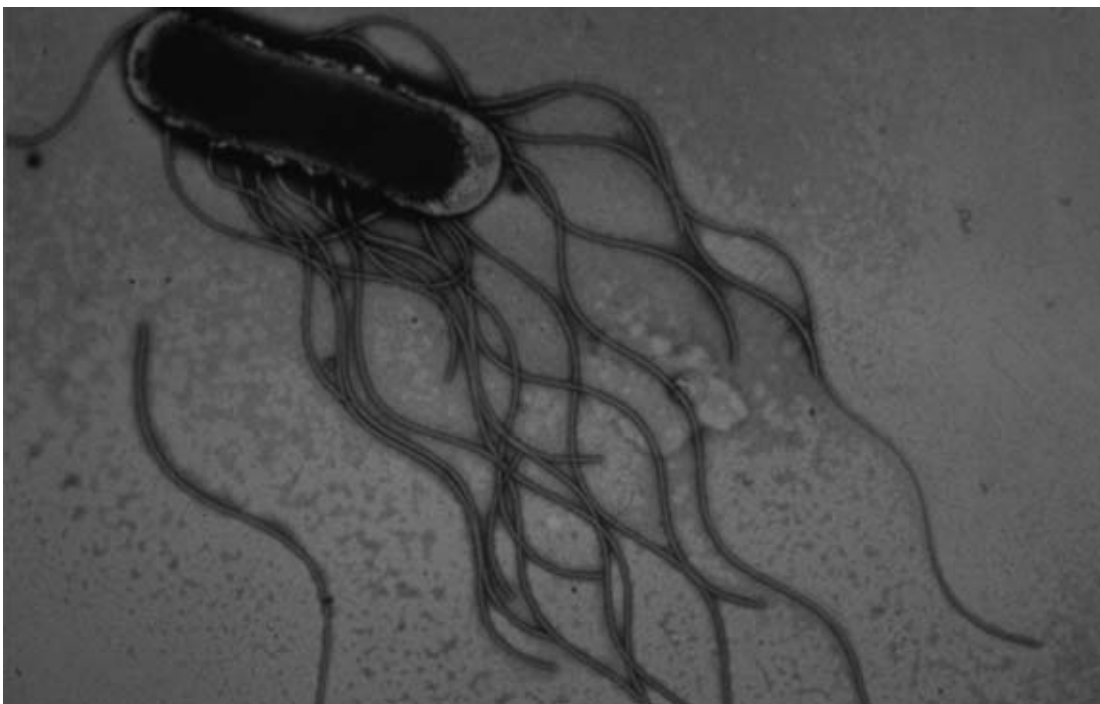
More recently, our laboratory has identified the A/J mouse as a suitable model for investigating gastrointestinal listeriosis. This mouse strain is not immune deficient, but does possess an innate susceptibility to infection with *L. monocytogenes* via various routes. We have confirmed that this is also the case when the organism is introduced into the gastrointestinal tract. Some of our data suggests that the organism may persist for a longer period of time in the g.i. tract in A/J mice than what is seen in more resistant strains of mice. This is somewhat reminiscent of the situation in human listeriosis, where the period of time between ingestion of *Listeria*-contaminated food products, and onset of disease, can be several weeks.

Furthermore, the A/J mouse provides a model one in which we can evaluate how growth of the organism on different food products affects its virulence in the gastrointestinal tract, without further manipulation of the host's immune system.

We have used this model to assess how growth of *Listeria monocytogenes* in packages of RTE meat can influence the resulting severity of the infection. We find that packages of wieners do not support multiplication of *Listeria monocytogenes*, presumably because of the addition of sodium lactate/ sodium diacetate, to the product. In contrast, smoked turkey slices, which lack sodium lactate/ sodium diacetate, support substantial growth of the organism at mild abuse temperatures. We also have some preliminary evidence suggesting that the severity of the infection may be greater, and perhaps more prolonged, in A/J mice inoculated with *Listeria monocytogenes* grown on turkey slices, than in bacteriological broth.

### Expected accomplishment

During the next period of support, we will continue our evaluations of *L. monocytogenes* grown on RTE meat products and screen transposon mutants being developed in the laboratory of our USDA collaborator Dr. Luchansky. These lack a global regulatory gene and some do not express flagella. This might result in alterations in their virulence in the gastrointestinal tract. We will assess this possibility by screening some of these mutants in the A/J mouse model described above. At the end of this study, we hope to have information that will help us better understand how different strains of *Listeria monocytogenes* vary in their ability to cause gastrointestinal disease, how innate defense mechanisms combat infection, and how growth on different food products might alter the virulence of *Listeria monocytogenes* in the gastrointestinal tract.



## The construction of microarrays for the transcriptional analysis of the genes of *Listeria monocytogenes*

F. Chris Minion (Iowa State University, Ames, IA)

### Major issue

The goal of this research is to develop a tool to be used to analyze gene expression of *Listeria monocytogenes* exposed to different environmental conditions. The research will generate microarrays on glass substrates that will be distributed to units of the National Alliance for Food Safety. They will use these arrays to analyze the transcriptional activity of the entire complement of listeria genes, the transcriptome, simultaneously under different environmental conditions. This is, in effect, a parallel analysis of differentially regulated genes.

The array is composed of polymerase chain reaction (PCR) products representing unique regions of each gene of the genome of *L. monocytogenes*. The product sizes range from 100-300 base pairs in length. To produce these it was necessary to first design PCR primers unique to each gene, perform the PCR reaction, quality control each reaction, purify the PCR product and quantitate each product.

### Relevance of problem

*Listeria* continues to be one of the important pathogens found in processed foods. Its ability to survive in various environments under strikingly different temperatures is a hallmark characteristic of this organism and possibly one of its key features in dissemination and disease. Our understanding of the genes and their regulation in regards to environmental changes is poorly understood. Also, little is known about the effects of different environmental insults on gene regulation.

We are seeking ways to improve diagnostics and therapeutic strategies to help control listeriosis at both the pre-harvest and post-harvest levels. Completion of these studies will enhance our fundamental understanding of the molecular interactions of *L. monocytogenes* in the environmental conditions encountered during its path from farm to food to human.

### Significant achievement

- The genome sequence of *L. monocytogenes* strain EDG (1/2A) was completed by the Institut Pasteur and was used as the foundation strain for the array. Subsequently, the 4b strain was almost completed by The Institute for Genomic Research and genes unique to that strain were added to the array.
- Each of the gene sequences was analyzed for PCR primers meeting certain standards of size, annealing temperature, and product size, and the resulting group of primers were tested against all other genes in the genome to ensure specificity.
- PCR reactions were performed with all PCR primer pairs and the cognate chromosomal DNA to give 2,777 products from strain 1/2A and 96 products from strain 4b.
- Preliminary experiments were performed to optimize the substrate manufacturer, the spotting buffer and the spotting conditions.
- Test slides were spotted and are presently being examined for spot morphology, content and hybridization efficiency.

### Expected accomplishment

Year 1

- Analyze the genome of strain 1/2A and design the PCR primers needed for the project.
- Compare genomes of strains 1/2A and 4b and determine the genes unique to 4b. Design PCR primers for those genes.
- Perform PCR reactions, purify products and quality control the reactions.

Year 2

- Complete the PCR reactions including those that failed to amplify on the first round.
- Perform initial spottings and test various substrates for consistency, background levels and other characteristics.
- For those genes, additional primers were designed and reaction conditions were optimized.

**Partnership**

We expect that these tools will be used in a variety of studies to better understand the role of gene regulation in persistence and virulence of the organism. We also expect that this tool can be used to study genetic variation in *Listeria* isolates to help predict the role of variation in disease. This will require the expertise and contributions of many USDA scientists. Specific partnerships, however, have been developed with Irene Wesley and John Bannantine (National Animal Disease Center, Ames, IA) and Todd Ward (National Center for Agriculture Utilization, Peoria, IL).



## Eliminating *Listeria monocytogenes* from packaged and refrigerated RTE poultry products

Rong Murphy, 203 Engineering Hall, BAEG, University of Arkansas, e-mail: rymurph@uark.edu, phone: 479-575-2542, fax: 479-575-2846

Mark Berrang, USDA-ARS-SAA-PPMQRU, e-mail: mberrang@saa.ars.usda.gov, phone: 706-546-3551, fax: 706-546-3633

### Major issue

The objective of this project was to determine the efficacy of in-plant heat pasteurization of packaged fully cooked poultry meat products to eliminate the human pathogen *Listeria monocytogenes*.

### Relevance of problem

Post-process handling is one of the primary causes of pathogen contamination in ready-to-eat (RTE) meat and poultry products. Unless a process is operated in an aseptic environment re-contamination can occur. To eliminate pathogens such as *Listeria monocytogenes* and ensure food safety, the deli RTE meat and poultry products can be pasteurized prior to or after packaging via steam or hot water.

The thermal inactivation of *Listeria monocytogenes* in different RTE poultry products was evaluated. The effect of product formulation on the thermal inactivation kinetics of *Listeria monocytogenes* was determined. From this research, process lethality of the product during post-cook pasteurization can be predicted via the temperature-time data collected in a process.

For in-package surface pasteurization of RTE meat or poultry products, heat needs to penetrate through a packaging film to reach the product surfaces. We determined the heating time needed for heat to penetrate through different thickness of the packaging films that were used to wrap RTE products. The relationship between heating time and pathogen reduction was also substantially affected by crevices, dents, cuts, folds, netting marks, cracks, wrinkles, and/or tears present on the product surfaces underneath packaging.

For sliced products such as chicken strips, the concern of pathogen contaminations on product surfaces will require every exposed surface to be treated. This will lead a time-consuming in-package pasteurization process by steam or hot water. After post-cook pasteurization, substantial cooling time was also required. The cooling time needed depends on the heat penetration depth in a product. Post-cook pasteurization time was also affected by cooker designs such as heat transfer coefficient and circulation rate or velocity. The results from our research will help the users to achieve a better process control to minimize deviations.

### Significant achievement

Studies were also conducted to determine the changes of physical properties for the pasteurized poultry products. No significant differences were found on water activity and instrumental texture (shear force) between treated and untreated products. However, significant differences were found in expressible and total moisture between treated and untreated products. These changes in physical properties could affect water-holding capacity and sensory characteristics in treated products.

### Expected accomplishment

From this research, a model was developed to correlate lethality of *L. monocytogenes* with process parameters during steam or hot water pasteurization. The results from this study should help processor develop a post-cook in-package heat treatment process for eliminating the pathogen from RTE meat and poultry products.

## Application of dielectric heating to kill human pathogenic bacteria on alfalfa seeds

<sup>1</sup>Dr. Larry R. Beuchat and <sup>2</sup>Dr. Stuart O. Nelson

<sup>1</sup>Center for Food Safety, University of Georgia and <sup>2</sup>USDA-ARS, Russell Agricultural Research Center, Athens, GA

### Major issue

Several documented outbreaks of enterohemorrhagic *Escherichia coli* O157:H7 and *Salmonella* infections in the U.S. have been associated with consumption of alfalfa and clover sprouts in recent years. Treatment of seeds intended for sprouting with chlorine (up to 20,000 ppm) and other chemicals reduces populations of these pathogens but does not eliminate all viable cells. The hypothesis is that cells of pathogens are lodged in cracks and crevices of damaged seeds, making them inaccessible to chemical treatments.

### Relevance of problem

Human listeriosis associated with consumption of seed sprouts has yet to be documented. However, *Listeria monocytogenes* is not uncommonly found in decaying vegetation and on healthy plant materials, so its presence in some lots of seeds intended for producing sprouts is likely. When present on alfalfa seeds, this pathogen is known to grow to populations exceeding 10 million per gram of sprouts produced. The ability of *L. monocytogenes* to grow at temperatures as low as 3°C (37°F) on a wide range of foods raises concern about its behavior on seed sprouts exposed to temperatures routinely used during distribution, marketing, and preparation in foodservice and home settings.

Dielectric heating, i.e., radio-frequency (RF) electric energy, has been successfully used to kill insects and molds in grains. Another important beneficial effect of dielectric heat treatment is that it stimulates germination of some types of seeds, including alfalfa seeds. In fact, much of the work done on dielectric heating treatment of alfalfa seeds has been directed toward determining treatment conditions necessary to decrease the “hard” seed content, with the objective of increasing germination percentage and enhancing vigor of plants in the early stages of development. Hard seed is a common condition found in alfalfa and many other legumes. Although seeds are viable, an impermeable seedcoat prevents the entry of water necessary to initiate germination. Hard seeds lie dormant in the soil or, in the case of sprout production, fail to germinate, thus reducing yield. Mechanical scarification, an abrasion of the seedcoat, is often used to reduce the hard seed condition. This process, however, undoubtedly exacerbates the problem of killing human pathogenic bacteria by application of chemical solutions because the active components of these solutions may not reach cells lodged at subsurface locations. The use of dielectric heating as a disinfection treatment would eliminate this problem, since temperatures at subsurface locations exceeding those required to kill human bacterial pathogens can be achieved within seconds.

Parameters influencing the effectiveness of dielectric heating in reducing the percentage of hard seeds include the radio frequency (MHZ) applied, field intensity (Kv/cm), temperature, moisture content of seeds, and time of exposure to treatment. High radio frequencies result in more rapid heating, thus influencing the time needed to achieve the desired reduction in number of hard seeds. The field intensity doesn't appear to affect changes in percentage of hard seeds, all other conditions being the same, but higher seed moisture content tends to diminish the effectiveness of dielectric heating. It was hypothesized that treatment of alfalfa seeds at a radio frequency of 39 MHZ for times sufficient to achieve temperatures in the range of 70 - 90°C (158 - 194°F), which greatly enhances the germination percentage of alfalfa seeds, would also substantially reduce or eliminate *E. coli* O157:H7, *Salmonella*, and *L. monocytogenes* that may be present on seeds.

**Significant achievement**

The objective of this project was to reduce populations of *E. coli* O157:H7, *Salmonella* and *L. monocytogenes* on alfalfa seeds by at least 100,000-fold while simultaneously enhancing germination and not adversely affecting vigor or sensory quality of the sprouts. The efficacy of dielectric heating in killing pathogens, as affected by moisture content of seeds and various treatment time/temperature combinations, was studied. The effect of treatments on seed germination percentage and vigor (appearance, turgor, color) was also investigated.

**Expected accomplishment**

The potential for controlling human bacterial pathogens on alfalfa seed used in the production of sprouts by dielectric heating was studied by experimental exposure of alfalfa seed artificially contaminated with *Salmonella*, *E. coli* O157:H7, and *L. monocytogenes* to RF dielectric heating treatments at 39 MHz and different electric field intensities for varying times of exposure. Moisture content of alfalfa seed and final temperatures produced by the RF exposures were determined, and control and treated seed samples were analyzed in the laboratory for reduction of bacterial populations and affects on seed germination. Significant reductions in populations of all three pathogens were achieved without reductions in seed germination, but desired levels of pathogen reduction were not achieved without significant damage to seed germination. However, treatments effective in significantly reducing bacterial pathogen populations also increased alfalfa seed germination through reductions in hard seed percentages, so the combined benefits should be considered in evaluating dielectric heating treatments for practical use.



## Role of nematodes in pre-harvest contamination of fruits and vegetables with pathogenic bacteria

<sup>1</sup>Dr. Larry R. Beuchat, <sup>2</sup>Dr. Phillip L. Williams, and <sup>3</sup>Dr. Patricia D. Millner

<sup>1</sup>Center for Food Safety and <sup>2</sup>Department of Environmental Health Science, University of Georgia, Athens, GA; and <sup>3</sup>USDA-ARS-BARC, Beltsville, MD

### Major issue

Outbreaks of human bacterial infections associated with consumption of raw fruits and vegetables in recent years have raised interest in identifying factors that increase the potential for contamination. Although free-living, bacterial-feeding nematodes are ubiquitous in soil in which fruits and vegetables are grown, little is known about their potential role as vectors of human pathogens.

### Relevance of problem

The production of organic fruits and vegetables has increased markedly in recent years and organic farming often involves the use of animal manure and manure compost to enrich the soil. It is known that free-living, microbiovorous nematodes such as *Caenorhabditis elegans* are attracted to bacteria present in manures and manure slurries. After ingestion by *C. elegans*, bacteria inside the gut may grow and be protected against environmental stresses. It has been reported that *Salmonella* can persist in the gut of *C. elegans* for at least 5 days. Persistence may be related to attachment of the pathogen to epithelial cells. Enterohemorrhagic strains of *Escherichia coli* are known to also bind tightly to cultured mammalian cells. Binding may enable *E. coli* O157:H7 to persist in the gut of *C. elegans*. A better understanding the ecology of foodborne pathogens in association with free-living nematodes and the role nematodes play as vectors of pathogens to contaminate fruits and vegetables eaten raw may lead to interventions to prevent contamination of produce.

### Significant achievements

Results of research conducted during the first 6 months of the project reveal that *C. elegans* and *Diploscapter* sp. are attracted to several strains of *E. coli* O157:H7, *Salmonella*, and *L. monocytogenes* on laboratory agar. Results suggest that *E. coli* O157:H7 and a multidrug resistant strain of *Salmonella* can attach to or colonize (removed) in the gut of *C. elegans*. Over time, undigested, unattached cells are excreted by the worms. If excreted into an environment that supports survival or growth, populations may increase. Worms may then consume these cells and the cycle is repeated. As the worms consume and digest these cells, fewer viable cells would be recovered from their gut. After the worms expire, any viable bacterial cells remaining in the gut may obtain nutrients from fluids released as the worm decays. Evidence that this occurs is provided by the increase in initial populations of all test bacteria in worms incubated on agar at 37°C. Temperature has a significant effect on populations of bacteria recovered from the gut of *C. elegans*.

### Expected accomplishment

To date, the study provides evidence that free-living nematodes may be carriers or vectors of pathogenic bacteria. Contact of *C. elegans* that had fed on pathogens with preharvest fruits and vegetables may result in contamination and consequent increase in risk of disease associated with their consumption. Studies are now focused on determining the behavior of free-living nematodes in soil and manure.

## Decontamination of alfalfa seeds and sprouts by ozonation

Ali Demirci, Larry R. Beuchat, Kay H. McWatters, and William F. Fett

### Major issue

Use of alfalfa sprouts in sandwiches and salads is popular in the U.S. as well as other countries. Since 1995, there have been numerous foodborne disease outbreaks due to consumption of sprouts. The great majority of outbreaks have been attributed to contamination with various *Salmonella serovars*, and two outbreaks have been attributed to *E. coli* O157:H7 or O157:NM. A wide range of liquid chemical sanitizers for sanitizing seeds was found not to be highly effective in killing pathogens, most likely due to their lack of penetration in an active form into cracks and crevices in the seed coat. Therefore, it is essential to develop new and highly effective methods to disinfect sprouting seeds and sprouts to minimize the risk of future outbreaks. The goal of this research was to investigate the effectiveness of ozone treatment to inactivate *Escherichia coli* O157:H7 and *Listeria monocytogenes* on alfalfa seeds and sprouts. The specific objectives were to:

1. Determine the most effective mode of introduction of ozone to kill bacterial pathogens on alfalfa seeds and sprouts.
2. Optimize dissolved ozone concentration and contact time.
3. Determine the effect of agitation during treatment.
4. Determine the effect of ozone treatment on microbial biofilms on sprouts.
5. Determine the effect of ozone treatment on sensorial qualities of sprouts.

### Relevance of problem

The consumption of raw sprouted seeds has led to a substantial increase in the occurrence of food poisoning outbreaks. The first foodborne disease outbreak from sprouted seeds was recorded in 1973 (Portnoy et al., 1976). Over the years, many such cases of illnesses have been reported worldwide. Between 1995 and 1998 there have been nine major *E. coli* O157:H7 and *Salmonella*-related outbreaks, in the United States, associated with commercial sprouts (NACMFC, 1999a).

Contaminated seeds have been the most likely source of pathogens in outbreaks associated with sprouts. The potential sources for seed contamination include irrigation water, use of inadequately treated manure as fertilizer, location of fields close to animal rearing facilities, and poor worker hygiene (NACMFC, 1999b). Researchers have tried to develop methods to eliminate pathogens on seeds without reducing germination percentage (Lang et al., 2000; Taormina and Beuchat, 1999a,b). Decontamination of seeds by treating with 20,000 ppm of chlorine (using calcium hypochlorite) is recommended by US FDA (Federal Register, 1999). However, in some cases, this treatment does not eliminate *E. coli* O157:H7 from laboratory-inoculated seeds (Taormina and Beuchat, 1999a) as evidenced by subsequent growth during sprouting.

In addition, organic farmers, who constitute about half the sprout growers, are reluctant to use this decontamination method (EH update, 1999). Thus, alternative methods for treatment of alfalfa seeds and postharvest treatment of sprouts are needed. Ozone is one chemical under investigation for inactivation of microorganisms on meat, poultry, eggs, fish, fruits, vegetables, and dry fruits (Kim et al., 1999). Ozone acts as a disinfectant in either the gaseous state or when dispersed in water. It is an effective biocide against virus, bacteria, bio-film, fungi, protozoa and some other higher forms of life such as worms and mites (DEL Agriculture, 2000). It offers a number of advantages over conventional chemical treatments with chlorine, sodium hypochlorite, and hydrogen peroxide. These include lack of residues or by-products, rapid dissociation back to oxygen, and reduced inhalation of disinfectant by user. Besides, it offers a non-thermal treatment suitable for seeds, sprouts, and leafy vegetables which are sensitive to steam and heat decontamination (FRPERC, 2001).

**Significant achievement**

We have investigated application of ozone to inactivate *E. coli* O157:H7 and *L. monocytogenes* on alfalfa seeds and sprouts. We have observed the followings:

- Up to 2.2 log<sub>10</sub> CFU/g reduction of *E. coli* O157:H7 on alfalfa seeds and sprouts with sparged ozone.
- Up to 4.8 log<sub>10</sub> CFU/g reduction of *E. coli* O157:H7 on alfalfa seeds with sparged ozone followed by heat treatment for 3 h at 60°C.
- Up to 1.5 log<sub>10</sub> CFU/g reduction *L. monocytogenes* on alfalfa seeds whereas, up to 1.3 log<sub>10</sub> CFU/g reduction *L. monocytogenes* on alfalafa sprouts.
- Ozone did not have detrimental effect on seed germination percentage and sensory quality of sprouts.

**Expected accomplishment**

We have achieved following outcomes during the NAFS grant:

- Inactivation of *Escherichia coli* O157:H7 on inoculated alfalfa seeds with ozonated water and heat treatment.
- Inactivation of *Escherichia coli* O157:H7 on inoculated alfalfa seeds with ozonated water under pressure.
- Inactivation of *Escherichia coli* O157:H7 on inoculated alfalfa seeds using pressurized ozone subsequent to creation of vacuum.
- Application of ozone for inactivation of *Escherichia coli* O157:H7 on inoculated alfalfa sprouts.
- Efficacy of ozone in killing *Listeria monocytogenes* on alfalfa seeds and sprouts, and effects on sensory quality of sprouts.
- Evaluation of native biofilms by confocal scanning laser microscopy.



---

## Future Plans for the NAFS

Future plans for the National Alliance for Food Safety include efforts in several key areas to maintain relevance in meeting the food safety challenges facing our nation. The priority food safety research areas of pre-harvest and post-harvest microbial systems will continue to be addressed by the NAFS. The objectives will be to strengthen the effectiveness of HACCP systems, design improved testing procedures, including statistical sampling plans, and novel intervention strategies for control of human pathogens. Research related to implementing performance standards, process control verification as well as risk assessment will also be ongoing. The NAFS leadership will continue to explore service opportunities working through the Virtual Centers in an effort to streamline and expand the effectiveness of the rapid response capacity of the NAFS. Rapid response is the key to addressing emerging challenges faced by many of our constituent groups, including consumers, government and industry. The NAFS will also continue to develop stronger working relationships with the USDA, FDA and other government agencies as part of their mission to protect the nation's food supply.

---

## Funded NAFS Projects

<i>University</i>	<i>Title of Grant</i>
University of Tennessee	Epidemiological associations of <i>E. coli</i> O:157 from produce, environmental samples and animals
Iowa State University	Effect of dietary vitamin Es on colonization of <i>L. monocytogenes</i> in live turkeys and the microbiological safety and storage stability of turkey breast meat.
Iowa State University	The impact of commercial pre-slaughter processes on prevalence of human pathogens in ground pork from culled sows.
Washington State University	Molecular epidemiological investigations of feed borne dissemination of <i>E. coli</i> O157:H7.
Iowa State University	Role of long polar fimbriae in intestinal colonization by <i>E. coli</i> O157:H7
University of Wisconsin	Prevalence and antibiotic resistance of <i>E. coli</i> O157:H7 in downer dairy cattle from the upper Midwest
Texas A&M University	Factors contributing to the presence of <i>Escherichia coli</i> O157:H7 in feedlots and feedlot cattle
University of Georgia	Application of dielectric heating to kill human pathogenic bacteria on alfalfa seeds
Pennsylvania State University	Decontamination of alfalfa seeds and sprouts by ozonation
Iowa State University	Microarrays for the transcriptional analysis of the genes of <i>Listeria monocytogenes</i>
Iowa State University	Irradiation and packaging treatments for controlling <i>L. monocytogenes</i> and improving sensory acceptability of ready-to-eat turkey breast roll.
University of Wisconsin	Dose response of infection following intragastric inoculation <i>L. monocytogenes</i> in ready to eat foods.
North Carolina State University	Novel virulence markers of food isolates of <i>L. monocytogenes</i> for rationale design of detection strategies
Smith/University of Georgia	Comparison of <i>L. monocytogenes</i> virulence in a mouse model for use in risk assessment
University of Arkansas	Eliminating <i>Listeria monocytogenes</i> from packaged and refrigerated RTE poultry products
North Carolina State University	Molecular ecology of <i>Listeria</i> , <i>Salmonella</i> and <i>Campylobacter</i> in the turkey processing industry
Michigan State University	Robustness of predictive models for <i>Listeria</i> growth and inactivation in ready-to-eat meat and poultry products
Iowa State University	Construction of microarrays for <i>E. coli</i> O157:H7
Scott/Texas A&M University	Transmission dynamics of antimicrobial resistance in integrated animal and human populations using molecular epidemiology
University of Georgia	Role of nematodes in pre-harvest contamination of fruits and vegetables with pathogenic bacteria
Auburn University	Effects of cool water washing of shell eggs on microbiological, interior quality, and environmental characteristics





Office of the Secretariat, Dr. Neville Clarke  
Texas A&M University  
College Station, Texas 77843-2129  
979-845-2855  
n-clarke@tamu.edu  
<http://nafs.tamu.edu>