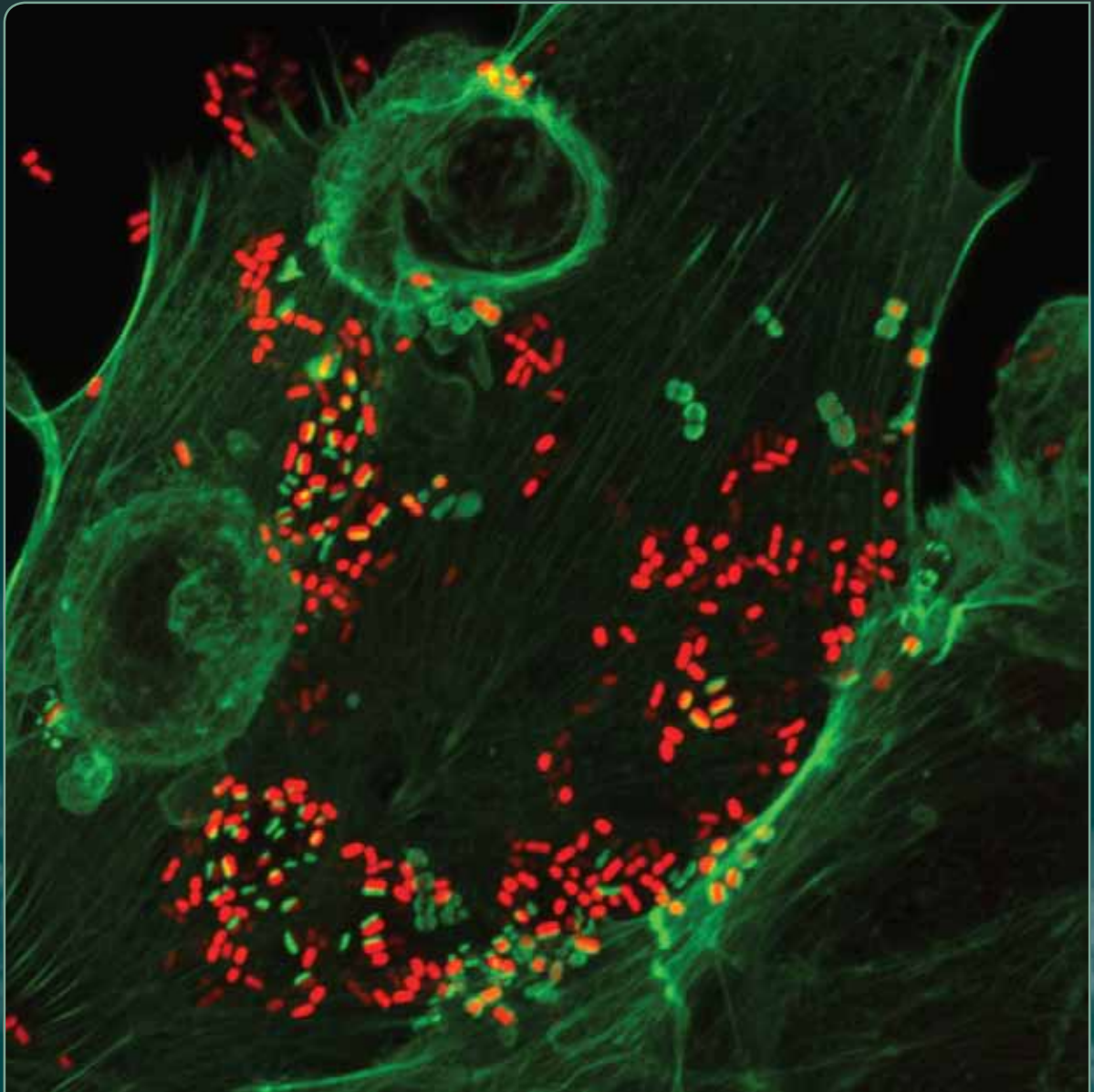


# Improving Beef Safety

**Shiga Toxin-Producing *Escherichia coli*: Prevention, Recovery, Characterization, and Control Across the Beef Chain**



Confocal laser scanning fluorescence micrograph of human colonic epithelial cells in culture infected with STEC O103:H2 bacteria (red), which have induced the formation of attaching-effacing lesions (green co-localized with bacteria).



Photo by STEC CAP PhD student, Zachary Stromberg, in lab of Director Dr. Rodney Moxley, University of Nebraska–Lincoln

# AFRI Grant Funds Multi-Disciplinary Effort to Improve Beef Safety

## Introduction

Shiga toxin-producing *Escherichia coli* (STEC) are a serious threat to the food supply and to public health, with more than 265,000 infections occurring in the United States each year. Most outbreaks are caused by ingestion of contaminated food or direct contact with fecal material from cattle or water and other ruminants.

A \$25 million grant coordinated through USDA's Agriculture and Food Research Initiative (AFRI) and administered through USDA's National Institute of Food and Agriculture (NIFA) was awarded to a multi-institutional, multi-disciplinary team of land-grant universities and government agencies led by the University of Nebraska-Lincoln. The team is conducting research and educating on how STEC contamination and outbreaks occur and spread throughout the beef production/processing chain, and on how science and technology can best be used to mitigate STEC risks. New and enhanced knowledge from this work will yield practical, effective information and communication tools to reduce STEC risk from beef.

A subset of seven STEC serogroups/serotypes, namely O26, O45, O103, O111, O121, O145, and O157:H7, have been declared adulterants in non-intact raw beef by the USDA Food Safety and Inspection Service. Additionally, another serotype, O104:H4, caused a large outbreak of foodborne illness in Europe in the summer of 2011. These specific serogroups/serotypes are the target of the STEC CAP grant and are hereafter referred to as STEC-8.

Considerable efforts are being made to reduce the risks associated with STEC in foods. The intestinal tracts of cattle are a major reservoir for STEC, with prevention of fecal contamination of carcass surfaces an important hazard control point at harvest. Since foodborne illness is predominantly caused by STEC-8, our researchers will focus on understanding and controlling the public health risks associated with these eight STEC serotypes (STEC-8) across the entire beef system.

The team's 50 investigators, from 12 U.S. institutions and other partners, bring to the project world-class facilities, broad expertise, existing program infrastructure, access to beef production and processing operations, and strong stakeholder support. The team ultimately aims to reduce human STEC cases and outbreaks from beef, while preserving an economically viable and sustainable beef industry. Their five main objectives, focusing on STEC-8, are detection, biology and characterization, intervention, risk analysis and assessment, and risk management and communication.

The projects described on the following pages meet these objectives.

Goals of this work will include to:

- develop and validate new, sensitive, and specific tests for the detection, isolation, and enumeration of STEC-8.
- define and mitigate risk from the most hazardous STEC serogroups/serotypes in the U.S. beef chain.
- learn under what conditions STEC are most likely to occur so precautionary measures can lower incidence of STEC and reduce the occurrence and/or duration of outbreaks.
- develop and validate methods to identify and control STEC during post-harvest processing.
- develop new and traditional communication approaches for effective message delivery to consumers, volunteers, restaurant staff, and other food handlers.
- use quantitative microbial risk assessment to integrate data and knowledge about the beef system to minimize health risks from all STEC strains.
- educate and modify behaviors of stakeholders across the beef continuum to provide immediate outcomes to significantly lower STEC exposure.
- bring real-world laboratory and field experience to students as future food safety professionals.

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*AFRI is NIFA's flagship competitive grant program established under the 2008 Farm Bill. AFRI's food safety grants promote and enhance the scientific discipline of food safety, with an overall aim of protecting consumers from microbial, chemical, and physical hazards that may occur during all stages of the food chain, from production to consumption.*

# Working as a Team to Control Shiga Toxin-Producing *E. coli* (STEC) in Beef

## Overview

### Project Goals

Reduce the occurrence and public health risks from eight targeted serogroups/serotypes of Shiga toxin-producing *Escherichia coli* (STEC) in beef using a microbial risk assessment platform.



Jill Hochstein, STEC CAP Project Manager



### STEC CAP Executive Management Team (EMT)

Rodney A. Moxley, University of Nebraska–Lincoln, Project Director

Harshavardhan Thippareddi, University of Nebraska–Lincoln

Randall K. Phebus, Kansas State University

John B. Luchansky, USDA Agricultural Research Service (ARS), Eastern Regional Research Center

Daniel L. Gallagher, Virginia Polytechnic Institute and State University

### Team of Participating Investigators (including EMT)

University of Nebraska–Lincoln – 12 investigators

Kansas State University – 20 investigators

North Carolina State University – one investigator

University of California–Davis – four investigators

University of Delaware – one investigator

Virginia Polytechnic Institute and State University – one investigator

New Mexico Consortium (Los Alamos National Laboratory) – two investigators

USDA-Agriculture Research Service – two investigators

New Mexico State University – one investigator

University of New Mexico – one investigator

Texas A&M University – four investigators

Mississippi State University – one investigator

Website: [stecbeefsafety.org](http://stecbeefsafety.org)





# Detection and Characterization of STEC

## Project 1

### Project Goals

Develop and validate cultural, immunological, and molecular methods for the detection, isolation, and enumeration of eight targeted serogroups/serotypes of Shiga toxin-producing *Escherichia coli* (STEC-8) along the entire U.S. beef chain.



### Team of Participating Investigators:

Rodney A. Moxley, University of Nebraska–Lincoln, Lead

Harshavardhan Thippareddi, University of Nebraska–Lincoln

T.G. Nagaraja, Kansas State University

Jianfa Bai, Kansas State University

Dick Oberst, Kansas State University

Sanjeev Narayanan, Kansas State University

Randall Phebus, Kansas State University

Alina Deshpande, New Mexico Consortium

Harshini Mukundan, New Mexico Consortium

John B. Luchansky, USDA Agricultural Research Service

Randall K. Phebus, Kansas State University

David Renter, Kansas State University

Ludek Zurek, Kansas State University

Anna Porto-Fett, USDA Agricultural Research Service

### Description

Microbiological, immunological, and molecular tools and methods for STEC-8 detection will be developed and validated, including rapid, high throughput screening assays that are compatible with current robotic sample processing systems, for use throughout the beef production and processing continuum. Tools and methods will include enrichment and agar plate culture media, monoclonal antibodies, immunological assays, multiplex polymerase chain reaction (PCR), real-time PCR, TaqMan assays, multiplexed oligonucleotide-PCR, waveguide-based optical sensors, and potentially others. These tests will be optimized, validated, and used to detect, isolate, and enumerate STEC-8 from feces, soil, water, feed, hides, flies, hot and chilled beef carcasses, primal and subprimal cuts, trim, purge from combo bins, and ground beef. Validation of each step in the detection, isolation, and enumeration process will be done on samples from the various matrices spiked with different concentrations and strains of STEC-8 and non-target organisms.

### Impacts

Development of new, sensitive, and specific tests for the detection, isolation, and enumeration of STEC-8, including rapid, high throughput assays, will improve the ability to detect and target antimicrobial interventions to reduce the incidence of STEC-8 throughout the beef chain and enhance the safety of the U.S. food supply.



# Prevalence of STEC Within the Beef Continuum

## Project 2

### Project Goals

Better understand how eight targeted serogroups/serotypes of Shiga toxin-producing *Escherichia coli* (STEC-8) impact the food supply chain by 1) determining the exposure risk of STEC-8 throughout the U.S. beef production system and 2) identifying the intrinsic or extrinsic factors that cause high rates of STEC-8 prevalence and beef product contamination.



### Team of Participating Investigators:

David Renter, Kansas State University, Lead  
Eric Wommack, University of Delaware  
David R. Smith, Mississippi State University  
Natalia Cernicchiaro, Kansas State University  
T.G. Nagaraja, Kansas State University  
Ludek Zurek, Kansas State University  
Terry Lehenbauer, University of California–Davis  
Sharif Aly, University of California–Davis  
Galen Erickson, University of Nebraska–Lincoln  
Terry Klopfenstein, University of Nebraska–Lincoln  
Rodney A. Moxley, University of Nebraska–Lincoln  
Michael Sanderson, Kansas State University

### Description

Studies of cattle, cattle production environments, beef processing plants, and beef products in multiple facilities across the country will be conducted in the summer and winter months. These data will demonstrate where and at what levels the eight targeted serogroups/serotypes of STEC occur in cattle and beef, and how levels of STEC-8 may vary among locations and over time. Beef processing plants and associated cattle production environments will be examined over multiple seasons to identify factors that contribute to STEC-8 transmission and beef contamination events. Beef cattle in multiple production systems will be studied to determine relationships among the cattle, environment, STEC-8, and animal management practices. Key factors associated with beef chain STEC-8 exposure will be identified, including frequency and concentration of live cattle colonization and contamination of cattle hides, beef carcasses and beef products. Genetic material from cattle and environmental samples will be used to characterize STEC-8 and other microbes in the cattle gut, on cattle hides, and in the production environment (e.g., water, feed, or insects). Isolated STEC-8 cultures will be studied to determine microbiological and molecular properties and vulnerabilities. Likewise, research will be conducted concurrently to recover and characterize STEC-8 from beef to better understand how to control these pathogens prior to human contact or consumption.

### Impacts

Learning under what conditions STEC-8 are most likely to occur and persist will help beef cattle producers and processors, as well as retail and food-service facilities take precautions to lower the occurrence and concentration of these pathogens thus reducing the risk of contaminated beef products reaching the public.

# Process Validation to Control STEC

Project 3

## Project Goals

Develop the scientific data, requisite documentation, and appropriate interventions necessary to create a food system that will reduce the risk of STEC in the beef supply.



## Team of Participating Investigators:

Randall K. Phebus, Kansas State University, Lead

John B. Luchansky, USDA Agricultural Research Service

Anna Porto-Fett, USDA Agricultural Research Service

Harshavardhan Thippareddi, University of Nebraska–Lincoln

Jeyam Subbiah, University of Nebraska–Lincoln

Amalia Yiannaka, University of Nebraska–Lincoln

Matthew Taylor, Texas A&M University

Alejandro Castillo, Texas A&M University

Gary Acuff, Texas A&M University

Nigel Harper, Kansas State University

Jim Cullor, University of California–Davis

Sean Fox, Kansas State University

Ted Schroeder, Kansas State University

Glynn Tonsor, Kansas State University

Rodney A. Moxley, University of Nebraska–Lincoln

## Description

Current and/or new commercial beef production and processing technologies applied before, during and after harvest will be assessed for their ability to reduce the risk of STEC-8. Biological, chemical, and physical antimicrobial intervention processes designed for carcasses, beef cuts, boneless beef trim, and ground beef, as well as veal products, will be evaluated alone and in series for their ability to control STEC-8 in raw and processed beef products. Large-scale inoculated studies will use common STEC-8 isolates and laboratory protocols across collaborating laboratories to facilitate accurate comparison of the effectiveness of different antimicrobial technologies. Bacterial isolates from beef products collected from processing facilities, as well as retail and food-service operations, will be characterized for their growth, survival, and/or destruction in response to food-relevant conditions for processing, handling, storing, and/or preparing beef. Traits that promote infectious disease and/or that allow these bacteria to persist or predominate along the beef chain continuum will be identified and further characterized. The cost and benefits for each practice will be evaluated to help beef processors of differing sizes decide how to best control STEC-8 in their beef processing operations.

## Impacts

Developing new and improving current antimicrobial technologies to control STEC-8 in beef production and post-harvest processing, and understanding how STEC-8 behaves under different conditions during post-harvest processing, will help beef producers and processors implement effective food safety management systems and enhance the safety of beef.

This research will generate validation data and supporting information that will immediately assist processors in supporting HACCP programs to control STEC-8 in beef and support Qualitative Microbial Risk Assessment (QMRA).

# Protecting Consumers of Beef from Shiga Toxin-Producing *E. coli*

## Project 4

### Project Goals

Gather real-world, food safety risk behaviors for consumer, retail, and food service arenas, and design interventions specific to target audiences that provide the highest return on resource investment, which can be evaluated using quantitative and qualitative data-gathering strategies.



### Team of Participating Investigators:

Ben Chapman, North Carolina State University, Lead

Doug Powell, Kansas State University

Christine M. Bruhn, University of California–Davis

Karen Cannon, University of Nebraska–Lincoln

### Description

Messages on safe food handling, preparation, and serving will be developed for several audiences, using various media, to prevent foodborne illness and enhance food safety from the eight serogroups/serotypes of Shiga toxin-producing *E. coli*; In the home, consumers often fail to wash hands before meal preparation and cross-contaminate through unwashed faucet handles. Another example is that less than 5 percent of consumers use a thermometer to check that cooked ground beef achieves the proper temperature of 160°F. For shoppers, allowing children to sit in grocery carts results in a higher incidence of foodborne illness in their households. Shoppers also need to be better able to understand beef product label statements to enhance product credibility. Education and training also are needed for individuals who serve food to large numbers of people, such as at church or community dinners. In restaurants, students trained as secret shoppers will ask servers in some 200 family-style restaurants about burger doneness, thermometer use, and food safety. The team also will explore the use of social media to teach food safety leading to behavioral change, and determine the effectiveness of this approach by monitoring behavior that occurs during a food safety crisis.

### Impacts

Traditional and new communication approaches, such as social media, infosheets, and other techniques, will improve food safety practices by consumers, volunteers, restaurant staff, and other food handlers and, in turn, may reduce the frequency, severity, and/or duration of foodborne illness from STEC-8.



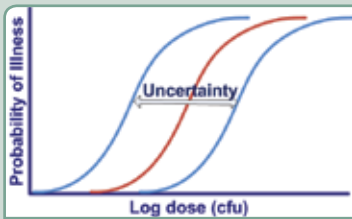


# Monitor and Mitigate STEC in the Beef Chain

**Project 5**

## Project Goals

Develop a farm-to-fork risk assessment on the threat of eight targeted serogroups/serotypes of Shiga toxin-producing *Escherichia coli* (STEC-8) in beef to improve understanding of consumer exposure to STEC, as well as develop and validate the interventions most effective to enhance beef safety.



## Team of Participating Investigators:

Daniel Gallagher, Virginia Polytechnic Institute and State University, Lead

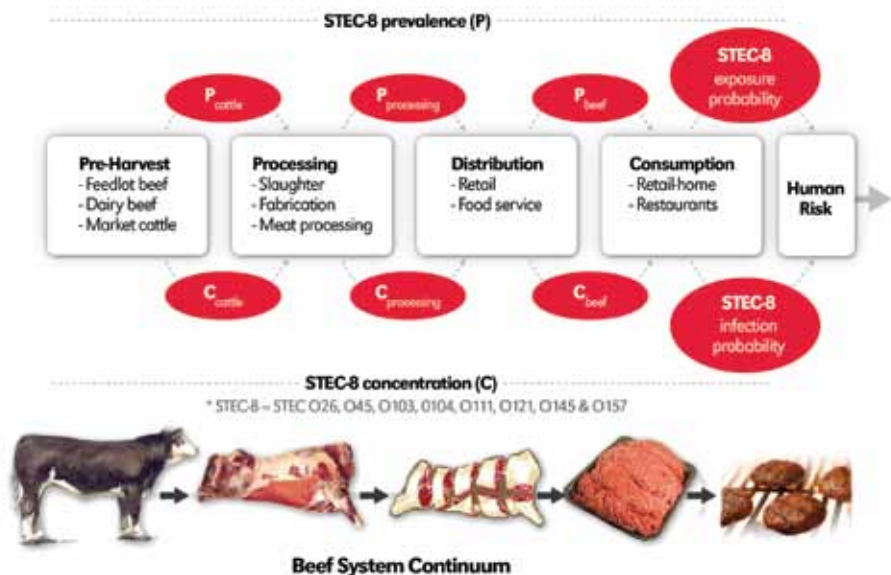
Michael Sanderson, Kansas State University

## Description

Quantitative microbial risk assessment (QMRA) is the most effective tool for managing microbiological hazards of foods; it serves as a transparent bridge between research and decision making. Input from scientists, economists, and industry will develop a risk assessment model that encompasses the complete beef supply chain. Limited availability and quality of data, such as a lack of STEC-8 diagnostic and detection capacity, limit the usefulness of QMRA. However, predictive analyses, such as the Process Risk Model (PRM), can help estimate the risk of pathogen prevalence and concentration throughout the food manufacturing process. Subsequent risk mitigation interventions to meet public health goals must address cost-benefit factors and risk-cost tradeoffs. A computer research model, representing a virtual food production and supply chain for STEC in beef, will integrate current knowledge of how STEC prevalence, levels, and types change during cattle and beef production and processing. Among other things, the model will identify current data gaps and uncertainties attributable to STEC as a foodborne disease while fostering the design of cost-effective strategies to protect public health.

## Impacts

Application of a QMRA will incorporate experimental data with comprehensive knowledge of beef production and processing, and lead to the development of economically, environmentally, and epidemiologically optimized interventions to minimize overall public health risks attributable to STEC. Data collected will significantly improve the ability to prevent and control STEC contamination, and identify research needs to support scientifically sound policy decisions and control efforts.





# Training the Current and Future Workforce and Consumers to Control STEC

## Project 6

### Project Goals

Establish a holistic food safety culture across all sectors of the beef food chain. This culture will be founded on the development, delivery, and validation of educational/training programs customized to various beef production/processing stakeholder groups.



### Team of Participating Investigators:

- Daniel Thomson, Kansas State University, Co-lead
- Curtis Kastner, Kansas State University, Co-lead
- Dann Husmann, University of Nebraska–Lincoln
- Karen Cannon, University of Nebraska–Lincoln
- Justin Kastner, Kansas State University
- Jason Ellis, Kansas State University
- Jeff Witte, New Mexico State University
- Dee Griffin, University of Nebraska–Lincoln
- Dennis Burson, University of Nebraska–Lincoln
- John Rupnow, University of Nebraska–Lincoln
- Harshavardhan Thippareddi, University of Nebraska–Lincoln
- Davey Griffin, Texas A&M University
- Ben Chapman, North Carolina State University
- Randall Phebus, Kansas State University
- Rodney Moxley, University of Nebraska–Lincoln

### Description

Libraries of industry-oriented, bilingual training modules for defined topics on beef production, processing, distribution, and preparation of safe products will be developed and offered online through Kansas State University’s Beef Cattle Institute. An optional trained worker certification database will be maintained, and on-campus and distance classes for degree programs and continuing education also will be offered. Kansas State’s Biosecurity Research Institute and its Food Science Institute will incorporate their work into educational formats. The University of Nebraska–Lincoln will develop a Web portal for information-sharing for stakeholders from producers through consumers. New Mexico State University will integrate minority students and faculty into our STEC CAP grant’s education and research efforts. Extension education nationwide will develop and offer STEC information and risk control tools to all stakeholder segments, and innovative products for 4-H and other youth. Beef processor training and guidance material for small companies will be a priority.

### Impacts

Development of a knowledgeable workforce and detailed food safety programs will ensure worker competency and attract the interest of future food safety scientists. STEC CAP education and outreach information developed and delivered to stakeholders spanning the entire beef system will yield immediate, positive, and relevant outcomes and behaviors that significantly lower STEC-8 exposure risks for consumers, and better protect public health.



# STECAP Student Internship/Externship Program, Workshop & Field Trip Experience

## Project 7

### Project Goals

Develop a competitive student training and education program (STEC-STEP) for students at the high school, undergraduate, graduate, and veterinarian levels that integrates field and laboratory research with university-level education. Training workshops will also be conducted for K-12 teachers to include food safety in their programs.



### Team of Participating Investigators:

Curtis Kastner, Kansas State University, Lead

Jeff Witte, New Mexico State University – Internship/Externship Program

Sarah Reasoner - Kansas State University – Internship/Externship Program

Justin Kastner, Kansas State University – Field Trip Experience

Jason Ellis – Kansas State University – Workshop

Dan Husmann – University of Nebraska–Lincoln – Workshop

### Description

Competitive internships for undergraduate, graduate, or veterinary/public health students will increase participants' knowledge of applied microbial food safety, animal health, meat/food processing, risk assessment, consumer sciences, and food safety education/communication. Interns will earn a stipend by working in the field and in the laboratories of STEC CAP grant investigators for a defined period. Fieldwork will be supplemented by discussions, technical writing, professional presentations, and performance evaluations. Upon completion, interns will present their achievements at the annual STEC CAP conference; subsequently, interns will be encouraged to apply for an additional sponsored field trip experience through the Frontier program at Kansas State University to learn about homeland security, food safety, food processing, agriculture policy development, etc. An externship program will bring 4-H, high school, junior college, and undergraduate students to the campuses of collaborating institutions to observe how science is being used to address significant food safety and public health challenges. Externship students will job shadow STEC CAP investigators in a laboratory or field setting for a period of one to two days. A defined number of internships and externships will be reserved for applicants from minority serving institutions. Workshops will also be conducted at the University of Nebraska–Lincoln and Kansas State University to educate K-12 teachers of the importance of integrating food safety into their curricula.

### Impacts

Bringing real-world laboratory and field experience to students will help spark interest in food-safety-related disciplines, and position them to effectively communicate the importance of scientific research related to food safety. These experiences may also serve as the catalyst for students to consider a career as a food safety professional.



# Participating Institutions



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United States Department of Agriculture  
National Institute of Food and Agriculture



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