Agricultural Research Service
National Program 108
Food Safety

2020 Project Accomplishments Report

Agricultural Research Service
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George Washington Carver Center, Beltsville, Maryland

Executive Summary

National Program for Food Safety (NP 108) is one of 15 National Programs (NP) within the USDA-Agricultural Research Service (ARS) Office of National Programs (ONP), and within Program areas: Nutrition, Food Safety and Quality (NFSQ). http://www.ars.usda.gov/research/programs.htm

NP108 is under USDA Strategic Goal 7. Provide All Americans access to a Safe, Nutritious, and Secure Food Supply. https://www.usda.gov/sites/default/files/documents/usda-strategic-plan-2018-2022.pdf.

NP108 is under Research, Education and Economics (REE) Goal 5, "Food and Nutrition Translation", and REE focus area "Food Safety and Health Promotion."

NP108 is under ARS Goal Area 1: Nutrition, Food Safety, and Quality; Goal 1.2 Protect Food from Pathogens, Toxins, and Chemical Contamination during Production, Processing, and Preparation; with Performance Measure 1. Develop new technologies that assist ARS customers in detecting, identifying, and controlling foodborne diseases associated with the consumption of animal products that affect human health.

For the Nation to have safe and affordable food, the food system must be protected at each step from production to consumption. The production and distribution system for food in the U.S. encompasses a diverse, extensive, and easily accessible system that is open to the introduction of biological and chemical contaminants through natural processes, global commerce, and intentional means. In response to these threats, crop and livestock production systems must be protected during production, processing, and preparation from pathogens, toxins, and chemicals that cause disease in humans. To ensure the security of production systems, NP108 conducts basic, applied, and developmental research resulting in new technologies, new and improved management practices, pest management strategies, sustainable production systems, and methods of controlling potential contaminants. These activities are key to providing a safe, plentiful, diverse, and affordable supply of food, fiber, and other agricultural products.

Mission Statement

To provide through research, the means to ensure that the food supply is safe for consumers and that food and feed meet foreign and domestic regulatory requirements. Research seeks ways to assess, control or eliminate potentially harmful food contaminants, including both introduced and naturally occurring pathogenic bacteria, viruses and parasites, toxins and non-biological-based chemical contaminants, mycotoxins and plant toxins. Food safety is a global issue; thus, the Program involves both national and international collaborations through formal and informal partnerships. Accomplishments and outcomes are utilized in national and international strategies delivering research results to regulatory agencies, commodity organizations, industry and consumers for implementation.

Vision Statement

To increase public health through the development of technologies which protect food from pathogens, toxins, and chemical contaminants during production, processing, and preparation thus increasing the safety of the food supply.

ARS 2016-2020 Action Plan for NP108 Food Safety

Goal: National Program (NP) 108*, Food Safety* through research, and in collaboration with regulatory agencies, industry, academia and other stakeholder and partners, provides the means to ensure that the food supply is safe for consumers and that food and feed meet foreign and domestic regulatory requirements. Food safety research seeks ways to assess, control or eliminate potentially harmful food contaminants, including both introduced and naturally occurring pathogenic bacteria, viruses and parasites, toxins and non-biological-based chemical contaminants, mycotoxins and plant toxins. Food safety is a global issue; thus, the research program involves both national and international collaborations through formal and informal partnerships. Accomplishments and outcomes are utilized in national and international strategies delivering research results and advances to regulatory agencies, commodity organizations, industry, academia, research and extension agencies and consumers.

In developing the 2016-2020 Action Plan, we approached the task appreciating that there are many special challenges, including: balancing stakeholder and partner needs with Program fiscal and personnel resources; ensuring that the research provides accomplishments that have impact and can be translated into practice through technology transfer; collaborating nationally and internationally and focusing on relevant issues to ensure that targeted areas are addressed; ensuring that the Program has the capability to respond when requested to unexpected needs and/or issues; and recognizing that the Action Plan remains a living document subject to review and realignment when and where required/appropriate.

Research Areas - 2016-2020 Action Plan

http://www.ars.usda.gov/research/programs/programs.htm?np_code=108&docid=278

Component 1. Foodborne Contaminants

Problem Statement 1 (A). Population Systems

Research Needs

- Improved approaches/designs for microbes based on population-based studies, monitoring of emerging pathogens, and supplying data for identified data gaps.
- Improved sampling collection protocols to maximize the probability of describing the exceedingly large number of diverse organisms that inhabit ecological communities.
- Data on the particular ecological niches or reservoirs for specific pathogens.
- Data on factors which enhance or reduce fitness characteristics related to persistent colonization, survival and growth.
- Data on the complex interactions between fungus/crop/environmental factors/production practices.
- Improved methods that allow evaluation of the impact of intervention or management strategies on microbial contamination throughout the entire food chain from field to plate.
- Data on how climate change impacts pathogen growth, persistence, pathogenicity or virulence.

Anticipated Products

- Improved epidemiological methods that allow the collection of quantitative data on the pathogen load within the food safety continuum.
- Capability to predict how environmental, nutritional, and/or biological factors influence or control the attributes and changes in ecological communities and within microbial populations.
- A foundation for developing appropriate intervention strategies based on mechanisms for transmission and dissemination of pathogens and toxins in and among food producing animals and crops.
- A risk-based framework that allows the integration of genomic data with disease outcome
- Descriptions of genetic traits associated with colonization and the evolution of virulence, including the development and movement of resistance genes, and the role of commensalism in resistance gene acquisition.

Potential Benefits/Impact

- Improves and enhances knowledge of how microbial populations in agriculture can potentially affect and impact public health.
- Delineates how microbial pathogens are transmitted and disseminated in and among food producing animals and plant crops (includes mycotoxin related research) allowing for future development of improved/alternate (environmentally compatible) intervention and/or control strategies.
- The critical factors which influence fitness characteristics related to microbial persistence colonization, survival, and growth allowing for future development of improved/alternate (environmentally compatible) intervention and/or control strategies.

Problem Statement 2 (B). Systems Biology

Research Needs

- Whole genome sequencing (WGS) of specific pathogens to provide data for developing high resolution genotyping and molecular serotyping methods, for identifying virulence attributes, and elucidating the differences between pathogens and non-pathogens.
- Identification and characterization of pathogen virulence factors and how they interact.
- Data to determine if and/or how virulence is directly related to the infective dose.
- Data on pathogen adaptive responses to intrinsic and extrinsic food stressors such as pH, a_w, temperature, O_2, and determine their role in pathogenicity and/or persistence.
- Data to determine if resistance genes affect virulence, pathogenicity and/or persistence.
- Identification and characterization of virulence attributes and the responses of specific pathogens to their environment relative to changes in immunogenicity in the host.
- A detailed investigation of food production and processing environments for bacterial pathogens, and a determination what genetic and/or environmental factors might determine or allow certain bacterial strains to become persistent.
- Data on the impact of changing management, production and intervention practices on the incidence of parasites as it relates to foodborne risk.
- Identification and characterization of unique fungal genes for specific biological and physiological functions. For example, how mycotoxin synthesis is transcriptionally regulated during the fungal growth cycle.
- Data on the effect of climate change on mycotoxin production in food crops. How environmental stress factors interact to affect plants, fungal growth, and subsequent mycotoxin synthesis.

Anticipated Products

- Identities of the critical/required genetic components that make specific microorganisms pathogenic versus non-pathogenic, or highly versus weakly virulent.
- Principles relating regulatory mechanisms that control or impact gene expression with a microorganism's biology, for example, pathogenicity and virulence.
- Information relating how stress factors such as climate change affect pathogen gene expression.

Potential Benefits/Impact

- Provides knowledge of which genes are required for a microorganism to become a pathogen; generates data on genes that contribute to variations in pathogenicity, and how gene expression is involved in virulence and/or persistence viability in animal, plant and food systems.
- Generates data for the specific development of molecular pathogen phylogenetics, allowing for improved and faster molecular tracking, and determination and characterization (attribution) of outbreaks of foodborne illness by regulatory agencies.
- Supports development of improved risk models, and the revision of risk assessments, e.g., pathogens of low virulence may not be considered as necessary for regulatory control.
- Supports improved mitigation strategies and alternative control measures via identification of genes that code for resistance to antimicrobials and disinfectants, for toxin production; for the ability to grow in specific ecological niches; and for the ability to persist in production and/or processing environments.

Problem Statement 3 (C). Microbial Contaminants: Technologies for Detection and Characterization

Research Needs

- Sampling protocols to maximize the probability of detecting contaminants especially when combined with innovative approaches to sample processing.
- Sample recovery methods with attention to sample preparation as different matrices may present unique problems.
- Methods that do not have a sample or detection bias.
- Technologies that have applications in surveillance systems, for monitoring the food supply and for food defense.
- Technology development that has uniformity of application in both pre- and postharvest production and processing system.

Anticipated Products

- Technologies for multiple agents for trace-back and attribution, and where fiscal and personnel resources are also limited.
- Technologies with improved speed, cost effectiveness, and the capability to provide information for the determination and implementation of subsequent actions.
- Validated technologies that allow uniformity of implementation nationally and internationally.

Potential Benefits/Impact

- Provides validated technologies that have public health, regulatory [monitoring, traceability and attribution], trade, industry, and research use and a commonality of interests between stakeholders and partners.
- Allows improved response times to events, and subsequently allows for the development of mechanisms for treating foods taken out of commerce.
- Provides data to identify areas where interventions are most critically needed, thus assisting the implementation of HACCP programs by Federal agencies, and their regulated industries.
- Enables development and validation of predictive microbial models and helps fill identified data gaps.

Problem Statement 4 (D). Chemical and Biological Contaminants: Detection and Characterization Methodology, Toxicology, and Toxinology

Research Needs

- Accurate, rapid, and easily used analytical detection methods: single/multiclass, single/multi-contaminant analytical methods; lab and field-based methods and instruments for analytical screening.
- Mechanism/action-based bioassays for laboratory and field use.
- Multi-task on/in-line inspection technologies that detect contaminants and quality attributes simultaneously functioning in or near real-time.
- Assays for assessing the efficacy of various processing methods to reduce or eliminate the toxicity in contaminated foods for human/animal consumption.
- Assays that have efficacy in toxico/toxinological studies.
- Intervention methods [bioremediation] to reduce bioavailability.
- Data on the fate and transport of contaminants and their derivatives in food systems and the environment for use in risk assessment by regulatory agencies.
- Provide parameters for regulatory agencies on biological residue depletion and withdrawal rates in animals.
- When requested, develop technologies that have a critical use in food defense.
- Data for use by regulatory agencies on the dose-response relationships and tissue specificity of biological toxins.
- Exposure assessment data for regulatory agencies on the relevance of biological toxins with undetermined toxicity through the use of animal models.
- Biomarker assays as a measure of exposure and disease susceptibility.

Anticipated Products

- New and validated technologies that when implemented provide tangible benefits through a more effective and efficient means of monitoring the food supply and environment where food is grown.
- Improved methods that assist researchers conducting toxico/toxinological studies.
- Toxico/toxinological data providing basic and applied knowledge on the effect of exposure to biological toxins.

Potential Benefits/Impact

- Provides technologies and data for regulatory use, and for better scientific and regulatory decision-making, reducing the likelihood of tolerance limit-errors, protection of consumers, and prevention of economic losses resulting from inappropriate regulatory actions.

Problem Statement 5 (E). Intervention and Control Strategies

Research Needs

- Interventions that prevent colonization or modulate pathogens in the gut; target specific metabolic endpoints; decrease shedding of zoonotic pathogens at the time of slaughter.
- Data on the role of transportation and lairage, slaughter/processing methods, and equipment on pathogen survival, transfer, post-harvest processing and storage.
- Data on the effect of intrinsic (pH, a_w, Eh, nutrient content, antimicrobials, structure) and extrinsic (temperature, RH, O_2) parameters in the production, processing, handling, preparation, and storage of foods. This need includes food preparation and handling for, or by food service operators and/or consumers.
- Data that elucidate the mechanism(s) of pathogen introduction, persistence/survival in shellfish.
- Production and processing intervention/control strategies for pathogen reduction in shellfish.
- For plant crops (fresh produce), obtaining data on the role of extrinsic and intrinsic factors on pathogen internalization and/or attachment; and pathogen occurrence and movement.
- For plant crops (fresh produce), obtaining data on the role and/or influence of commensals and/or non-pathogens.
- Identification of the critical control points in both production and processing of fresh produce, plant crops (grains/tree nuts) that can be mitigated through the development and implementation of intervention and control strategies.
- Biological control strategies to reduce mycotoxin production and contamination of food and feed crops such as corn/maize, cotton seeds, grains and tree nuts. Any new or modified effective biocontrol organisms and delivery systems must not introduce other toxic factors; for example, for the biocontrol of aflatoxins there should be no introduction or expression of the CPA or fusarium toxin genes.
- Data that assesses the role of chemicals that might act synergistically to enhance accepted interventions.
- Methods to prevent the growth of pathogenic and spoilage microorganisms in minimally preserved, brined, and fresh-cut foods.

- Data on the effect of single and/or combinations of intervention technologies on pathogen reduction. Validate these data through laboratory, pilot-plant processing and commercial processing facilities.
- Data on whether combinations of non-thermal technologies can be incorporated in the hurdle concept; and determine whether single or combinations of non-thermal technologies are more effective if used in combination with traditional interventions.
- Data that evaluates the outcome/impact of intervention options for small and very-small regulated plants.
- Data in intervention effectiveness to be for use in the development of Quantitative Microbial Risk Assessments (QMRAs)
- Data determining the effect of intervention technologies on sensory/quality deterioration, and accumulation of toxic chemical by-products.

Anticipated Products

- Improved intervention strategies to eliminate and/or control microorganisms in animals and their derived products, seafood and plant production, processing and storage systems. Interventions have the ability to inactivate microorganisms to varying degrees; therefore, the goal is to maximize intervention effectiveness while minimizing sensory/quality deterioration, and possible accumulation of toxic chemical by-products.
- Improved intervention strategies for various sized operations, utilizing environmentally compatible technologies.
- Improved intervention strategies focusing on the use of combinations of new or innovative technologies for (minimal) processing, thus mitigating the potential for the development of resistance.
- Improved interventions based on an understanding of their modes of action and effects on the microbial ecology of a food product, since inadequate suppression of spoilage could create an opportunity for human pathogen growth and toxin production.

Potential Benefits/Impact

- Provision of critical intervention strategy data to regulatory/action agencies, industry, and commodity organizations that allows for the development, evaluation, and implementation of Good Agricultural Practices (GAPs); Good Manufacturing Practices (GMPs) or regulations based on sound science.
- Enables methods/strategies for the evaluation of any developed interventions and controls.
- Provides production control interventions that reduce downstream contamination, which subsequently reduces disease risk.

Problem Statement 6 (F). Predictive Microbiology/Modeling; Data Acquisition and Storage; Genomics Database

Research Needs

Modeling

- Models that include an emphasis on probabilistic modeling to balance the deterministic approaches. This includes the influence of challenge strain(s); assessment of a model's performance; predictive value on extrapolation; and efficacy especially in complex food matrices where the intrinsic and extrinsic parameters may change.
- Data that examines and determines if growth/no-growth interface models predict the probability of growth occurring when a population faces more than one stressor/constraint.
- Models that have utility for risk assessment from both the producer and consumers perspective. There are distinctly different consequences of conservative (over) vs. non-conservative (under) prediction of growth or risk.
- Data that determines if changes in the microorganism(s) themselves occur, due to up/down regulation of genes; quorum sensing; or transfer of genetic information between species.
- Models that predict pathogen and non-pathogen behavior in complex food systems utilizing inactivation data. These types of studies are fundamental to developing Hazard Analysis Critical Control Point (HACCP) systems and regulations.
- Process risk models for industry that derives predictions for Critical Control Point (CCP) assessment.
- Data that demonstrates how models can be integrated more fully into supply chains, thereby increasing utility to industry and risk assessors.
- Models that determine the effects of food safety interventions, for example carcass and produce sprays; and physical and chemical interventions, for example: radio frequency, heat, cold, irradiation, and Generally Recognized as Safe (GRAS) chemicals.

Database

- Compile modeling data into a shared informational database through national/ international efforts.
- Write program code linking ComBase records to online databases. This feature will collect attributes for individual records, such as journal article title, abstract, authors, and institution.
- Data collection for specific organism-food combinations, enhance the value for the food systems community. Prioritization will be given to data needed to fill current database gaps, as well as records most sought after by ComBase customers.
- Derive and provide relevant data to regulatory agencies for use in HACCP programs, risk assessments, labeling, persistence, and issues relative to international trade.

Genomics

- Conduct sequencing and annotation efforts on pathogens of concern that fall under research efforts in various Problem Statements.
- Development of a genomic database for identification of microorganisms or development of an identifier of microorganisms as a platform for storing data.
- User friendly system to aggregate, maintain, share, mine and translate genomic data for microorganisms, for example: the identification of relevant genes or for the comparison of genomes to detect outbreaks and emerging pathogens.
- Increased focus on bioinformatics (computational biology) as more sequence data becomes available, and the complexity of both the data and questions being asked become more sophisticated.

Anticipated Products

- Predictive microbiology [models] that have validity and usefulness while addressing the limitations of the predictive ability. Studies leading to development of these models will include "real food systems" not just broth models or model food systems.
- A shared informational database done in-part through the continued development and expansion of the international collaborative project Combase. This will include data from industry/academia that pertains to "real food production/processing systems."
- A computer-based system and database to aggregate, share, mine and translate genomic data for microorganisms in real-time through a direct link using user-friendly platforms.

Potential Benefits/Impact

- Generates data on the responses of microorganisms to both defined and changing environmental conditions, and translates these data into mathematical models and user friendly software tools available on the internet at no cost. These must be readily usable by national and international regulatory and public health agencies, and industry, to assist in ensuring the safety of the food supply.
- An internet-based database ensures that data-mining and acquisition will continue to be coordinated. Genomic database and bioinformatics efforts become increasingly important so that biologists have the ability to gain information that will foster technological innovation, and an understanding of the genetic basis of foodborne microorganisms.

Problem Statement 7 (G). Antimicrobial Resistance

Research Needs

- Multidisciplinary approaches to understand the development, persistence, and transmission of resistant genes, and antimicrobial resistant in foodborne microorganisms.
- Improved detection methods to assess bacteria for antibiotic resistance genetic elements in foodborne pathogens.
- Methods to assist other Federal agencies in measuring and assessing AMR in food animal populations, e.g., assisting FSIS in interpreting National Antimicrobial Resistance Monitoring System (NARMS) results and provide support for USDA's National Animal Health Monitoring System (NAHMS) studies on AMR bacteria.
- Alternatives to antibiotics including management practices, pre-and probiotics, bacteriophage gene products, lytic enzymes, vaccines and other novel products to reduce their levels in food producing animals, thus reducing the need for antibiotics. The development of any practice/product must ensure practicality and potential utilization so that implementation is cost effective to the producer, readily approved by regulatory agencies and industry, and easily incorporated into any management system.
- Elucidating the ecology of foodborne AMR bacteria in terms of gene transfer, the role of the host microbiome in the development and maintenance of AMR, and the role of biofilms in the development of AMR.

Anticipated Products

- Improved detection techniques facilitating the speed, ability, and accuracy of detecting foodborne AMR bacteria in food producing animals and their products.
- Improved strategies to reduce antibiotic use and the number of AMR bacteria in the food supply.

Potential Benefits/Impact

- Provides support for both stakeholders and regulatory agencies in developing strategies to address foodborne AMR bacteria.
- Improves strategies to reduce the use of antibiotics in production animals while maintaining their health and growth efficiency. This is critical for feeding an ever growing population while also addressing a serious public and animal health concern.

ARS locations addressing the 7 Problem Statements: Beltsville, MD; Wyndmoor, PA; West Lafayette, IN; Peoria, IL; Ames, IA; Albany, CA; Maricopa, AZ; Clay Center, NE; Fargo, ND; College Station, TX; Fayetteville, AR; Stoneville, MS; New Orleans, LA; Dawson, GA; Athens, GA; Raleigh, NC. Funded collaborations were also conducted with the Center for Food Safety Engineering, Purdue University, and the Mississippi Center for Food Safety and Post-Harvest Technology, Mississippi State University.

Significant Accomplishments

A mega-method to analyze contaminants in foods. Analysis of foods for the presence of pesticides, veterinary drugs, and environmental contaminants is necessary for public health. In 2003, ARS developed the QuEChERS approach to sample analyze pesticide residues in foods. This method has now become the primary and gold standard used worldwide in chemical residue analysis. Instrumentation and technology have continued to improve in the past 17 years, creating a need to update the QuEChERS method. Consequently, ARS scientists in Wyndmoor, PA, have now developed and validated the "quick, easy, cheap, effective, rugged, safe, efficient, and robust" (QuEChERSER) mega-method using mass spectrometry to analyze pesticides, veterinary drugs, and environmental contaminants in foods. So far, the new QuEChERSER mega-method has been validated for up to 349 diverse analytes in fish, bovine, caprine, and ovine muscle; hemp products; and fruits and vegetables. Once implemented internationally, QuEChERSER is expected to eventually supplant QuEChERS as the primary method for monitoring a wide array of chemical contaminants in foods.

Colistin-resistance in U.S. animal-origin food. Colistin, also known as polymyxin E, is a last-resort antibiotic against drug-resistant Gram-negative bacterial infections. Recently, a mobile colistin resistance gene, *mcr-1*, was discovered in clinical and animal samples. The prevalence of *mcr-1*-mediated colistin resistance has never been investigated in U.S. animal products. To fill this gap, ARS researchers in Albany, CA, screened more than 5,000 domestic food samples (chicken rinse, ground beef, beef trim, poultry, raw pork, and catfish) randomly collected by the USDA Food Safety Inspection Service for the presence of *mcr-1* using a novel method developed by ARS that combines an enzyme-linked immunosorbent assay with real-time polymerase chain reaction methods. The screening data revealed a very low prevalence (0.02 percent) of the *mcr-1* gene in tested samples. Subsequent whole genome sequence analysis on the single positive isolate revealed that the *mcr-1* gene resided on an IncI2 plasmid. This study was the first systemic and large-scale investigation of mobile colistin resistance in U.S. food animal products, and the information will be important for trade-related food safety risk assessments.

A novel strategy for estimating *Salmonella* contamination levels in raw ground beef. *Salmonella* is a leading cause of foodborne illness worldwide. In spite of the use of numerous process controls in food production industries, there has been little progress in decreasing the occurrence of *Salmonella* food poisoning over the past decade. This is in part because current testing methods indicate only the presence or absence of *Salmonella*, but they do not measure how much contamination is in a tested product. To address this need, ARS scientists in Clay Center, NE, developed a novel strategy for rapidly estimating *Salmonella* contamination levels in raw ground beef based in the same amount of time that it takes to detect *Salmonella* in enrichments using two different commercial molecular detection methods. The ability to detect high levels of *Salmonella* contamination will enable meat companies to improve their process controls and remove more highly contaminated products from the food chain. This will improve the safety of beef and decrease the incidence of human exposure to levels of *Salmonella* that cause disease.

Predictive models to identify antifungal compounds. Antifungal chemicals are often used to reduce crop spoilage and reduce the occurrence of mycotoxins. However, safer, better antifungal agents are needed. Some potential antifungal agents are phenolic compounds that have many uses due to their consumer-friendly properties. To aid in the selection of better antifungal compounds, ARS scientists in Peoria, IL, applied computational artificial intelligence and machine learning methods to develop mathematical

models that identified chemical properties of phenolic compounds that reduce contamination by mycotoxin-producing fungi. Two of the antifungal compounds evaluated, thymol and carvacrol, are components of essential oils of many plants, including the popular culinary herb thyme. These models will help toxicologists, microbiologists, and chemists discover better antifungal agents to benefit the food industry.

Survival of bacterial pathogens in manure-amended soils. Fresh produce, especially leafy greens, have been implicated as sources of several major outbreaks of foodborne illness in the United States. Manure-amended soils continue to represent a potential source of bacterial pathogen contamination. The Food and Drug Administration (FDA) Produce Safety Rule, as part of the Food Safety Modernization Act, prohibits the use of untreated manure within 90 or 120 days prior to the harvest of edible produce crops. To examine the role of manure types in produce contamination, ARS scientists in Beltsville, MD, in collaboration with the FDA and academia, collected manure/produce data from 12 field trials conducted over 4 years at 3 separate locations. The data were used to identify factors affecting the survival of *Escherichia coli* in manure-amended soils. The studies showed that poultry litter supported longer survival of *E. coli* than dairy or horse manure. Days of rainfall and soil moisture content affected *E. coli* survival in manure-amended soils. These results assisted the FDA in developing models to determine the appropriate interval between application of raw manure and harvest of edible crops to minimize fresh produce contamination, thus making produce safer for consumers.

Safe use of chlorine dioxide to sanitize produce and eggs. Chlorine dioxide (ClO_2) gas is very effective at eliminating microbiological contaminants from a variety of fruits, vegetables, melons, seeds, and even eggs. Although the gas has been proposed for sanitizing human foods to eliminate pathogens and rot organisms, it has not been approved by the Food and Drug Administration or Environmental Protection Agency for use on foods other than tomatoes and cantaloupe. This non-approval is because chemical residues of ClO_2 have not been described. ARS researchers in Fargo, ND, demonstrated that nearly all the residue deposited on the surfaces of eggs, avocados, onion, and sweet potato after the use of ClO_2 for sanitation is a harmless chloride ion. The studies also found that chlorate, a byproduct of ClO_2, was present in low quantities and could serve as a useful marker of ClO_2-treated products. The results show that chemical residues are not a major obstacle for the commercial development of cost-effective ClO_2 gas technologies to safely eliminate pathogens and rot organisms from a variety of produce and eggs.

Combining cold plasma and hydrogen peroxide as a postharvest intervention. Fresh fruits and vegetables are a major source of essential nutrients for humans. However, these products are subject to contamination by both pathogens and spoilage microorganisms, which reduces their safety, quality, and nutritional value. Fresh produce is often consumed raw or after minimal processing, and pathogen contamination can present higher risks of outbreaks of foodborne illness. Because there are food safety uncertainties along the supply chain, postharvest treatments are essential in reducing the risk of pathogen contamination and minimizing the risk of microbial spoilage. ARS scientists in Wyndmoor, PA, combined cold plasma and hydrogen peroxide aerosols to produce highly reactive radicals that reduced the populations of bacteria on fresh fruits. Applying cold plasma to hydrogen peroxide increased the effectiveness of hydrogen peroxide aerosols and killed almost 100 percent of *Salmonella* and *Listeria* on surfaces of apples, cantaloupe, and tomatoes. This new process did not affect appearance, color, texture, or nutritional quality of the produce. The outcome of this work has a direct application to the produce industry, and ARS is collaborating with industry partners to evaluate the commercial implementation of the new intervention technology.

A novel aqueous ozone intervention against *Escherichia coli* O157:H7 on fresh beef. Ozone is a naturally occurring water-soluble gas that is an effective germicide and has been approved as a sanitizer for food-contact surfaces and food products. *E. coli* O157:H7 is a foodborne bacterial pathogen that has been implicated in many cases of meat-associated human illness in the United States. The last step in beef processing is to rapidly cool the carcass to 35°F, and this is accelerated by applying periodic sprays of cold water. ARS scientists in Clay Center, NE, evaluated a new nanobubble technology that creates a stable, high concentration of aqueous ozone for its effect on pathogenic *E. coli* that can be present on beef during spray chilling. The results indicated that the novel ozone spray was 80 percent more effective in reducing pathogenic *E. coli* than water alone. Because beef carcasses are usually chilled under recurring sprays of water for 6–8 hours, by adding ozone this process can now be a continued antimicrobial step leading to safer end products for consumers.

A rapid test for masked toxins in wheat. Trichothecenes are a group of fungal toxins (mycotoxins) that can contaminate oat, wheat, barley, and corn, and cause substantial economic losses worldwide. Trichothecenes are toxic to humans and animals, and upon consumption, the toxin inhibits ribosomal protein, DNA, and RNA synthesis; mitochondrial functions; and cell division while simultaneously activating a cellular stress response. As part of efforts to improve monitoring of these trichothecene toxins, ARS scientists in Peoria, IL, in collaboration with the Institute of Sciences of Food Production in Bari, Italy, developed a new method to detect trichothecenes in wheat. Trichothecenes are also toxic to plants, but plants can protect themselves from the toxins by attaching a sugar residue to a trichothecene molecule, which makes them less toxic. The plant toxin derivatives are called masked mycotoxins, and are difficult to detect. During the human digestion process, the original toxin may be released from the masked state, resulting in mycotoxin poisoning. The new toxin/masked mycotoxin detection method is rapid, sensitive, and convenient and will be used to monitor trichothecenes and their modified forms in wheat. Improved monitoring for the trichothecenes and their masked forms can be used to reduce exposure to these toxins by diverting the contaminated food product from the food supply.

Wireless, high-resolution, time-temperature measurement of foods. Foods, especially ready-to-eat foods available for consumers via retail outlets, may undergo temperature fluctuations due to faulty refrigeration. These fluctuations may induce growth of pathogens and spoilage microorganisms. Continuous temperature monitoring is required to avoid any food safety or quality concerns. ARS-funded scientists at the Center for Food Safety Engineering at Purdue University in West Lafayette, IN, developed a system that can be integrated in delicatessen cases and is capable of acquiring temperature measurements using low-cost tags that can be attached to food packages. The system provides high-resolution temperature measurement that can be integrated into the "Internet of Things" (IoT) through Bluetooth communication capabilities. Integrating the system in retail deli cases can enable real-time risk assessment of stored products with direct notification of the management when irregular storage conditions occur. Implementation of such a system will enhance the safety and quality of ready-to-eat foods.

Flowing steam decontamination of broiler transport cages. Live-haul cages are used to transport broilers from the farm to a processing facility. These cages are large and expensive; therefore, companies have a limited supply and continually reuse the same cages. *Campylobacter*, a significant human foodborne pathogen, can be readily detected in the feces of broilers from a *Campylobacter*-positive flock. Feces left in a cage by broilers that carried *Campylobacter* can contaminate the next broilers placed in the same cage. Studies have previously shown that water spray and sanitizing broiler transport cages is logistically complicated, physically difficult, water intense, and largely ineffective to eliminate *Campylobacter*. ARS researchers in Athens, GA, tested steam as a means to decontaminate

transport cage flooring, which resulted in an approximately 99 percent reduction in the number of *Campylobacter* bacteria detected. When the steam treatment was preceded by a 15-second water spray, the *Campylobacter* reduction was improved to 99.99 percent compared with untreated cages. Although *Campylobacter* was not completely eliminated, steam shows potential as an effective method for sanitizing broiler transport cages and to control transfer to previously negative broilers. Lowering the number of *Campylobacter* bacteria on live broilers entering the processing plant would be expected to lessen contamination of fully processed poultry meat products and reduce consumer exposure to *Campylobacter*.

Eliminating *Campylobacter* in chicken livers. *Campylobacter* species are significant human foodborne bacterial pathogens specifically associated worldwide with poultry and poultry products. Foodborne outbreaks of campylobacteriosis, the disease ascribed to the pathogen, can often be traced to pâté or mousse prepared from undercooked chicken liver. *Campylobacter* is readily detected on fresh raw chicken livers in the processing plant and at retail. Infections have become so prevalent in the last several years that the USDA Food Safety and Inspection Service was prompted to call for interventions to decontaminate chicken liver. ARS researchers in Athens, GA, tested heat and cold treatments to lessen *Campylobacter* contamination of fresh chicken livers. Immersion in 60°C water for 5 minutes resulted in significantly lowering *Campylobacter* numbers. Forty-eight hours at −25°C in a household freezer was moderately effective. When heat and freezing were combined in series, a nearly 99 percent decrease in the number of naturally occurring *Campylobacter* on both the surface and within inner tissue of chicken liver was achieved. Thus, a mild heat process followed by freezing can be recommended for presentation of poultry livers at retail or home and will reduce consumer exposure to *Campylobacter*.

Simple and portable test for amatoxins. Amatoxins are lethal toxins found in certain mushrooms, in particular the death cap mushroom, which cannot be eaten. Because many inedible mushrooms are physiologically similar to edible mushrooms, many people (and animals) are sickened (and many die) from mistaken consumption. Additionally, most medical and veterinary personnel have difficulty determining which poison a human or animal might have ingested. ARS researchers in Albany, CA, developed an antibody-based immunoassay that can detect as little as 10 ng/ml of amatoxins in both mushroom and urine samples without the need for specialized equipment. The detection of amatoxins in urine samples correlated very well with the traditional methods of using liquid chromatography-mass spectrometry. The speed of analysis and lack of needing trained personnel and expensive instrumentation will offer a quick way to directly diagnose amatoxin-specific mushroom poisonings. The new immunoassay is currently being used and tested by several animal clinics through material transfer agreements and informal collaborations. A patent is pending for the new immunoassay.

Impact of agricultural runoff on Shiga toxin-producing *Escherichia coli*. Shiga toxin-producing *Escherichia coli* (STEC) are a group of foodborne bacterial pathogens that can be transmitted to humans mainly through food and water. STEC strains are implicated in more than 270,000 cases of human illness annually in the United States. STECs naturally reside in cattle, and are found in natural creek sediments that are affected by runoff and fecal pollution from agricultural and livestock practices. ARS researchers in Albany, CA, detected STECs from the water-sediment interface of two creeks in the Salinas River Valley of California, an area that is known to be associated with STEC-derived foodborne illness. Shiga toxin-encoding genes were not directly detected in the metagenomes of samples that were culture-positive for STEC, indicating that STEC was present at very low levels in those sediments. Furthermore, there were no significant differences in the abundance of human or cow-specific microbiome sequences between the control and sampling sites, implying a natural dilution of the

human inputs. This study provides metagenomic parameters for the Food and Drug Administration to use in enforcing the Produce Rule within the Food Safety Modernization Act.

Vaccination of cattle and impact on intestinal microbiota. Vaccines targeting the bacterial pathogen *Escherichia coli* O157:H7 (O157) in cattle have the potential to reduce O157 colonization and thus reduce carcass contamination. However, non-O157 *E. coli* are part of the normal microbiota, and vaccination against O157 *E. coli* may impact numbers and types of normal bacteria (microbiota) found in cattle intestines. Because the intestinal bacteria play a critical role in animal health, alterations induced by vaccination could affect an animal's immune response and overall health. ARS researchers in Ames, IA, evaluated the impact of O157 vaccination and O157 colonization on the diversity of intestinal microbiota to gauge potential unforeseen consequences of O157 vaccination. Microbiota analysis of fecal samples (which contains intestinal bacteria) from vaccinated and nonvaccinated cattle indicated a significant correlation between vaccination and alterations in intestinal bacterial populations. Whereas vaccination may be a strategy to limit O157 in cattle, unforeseen consequences of changes in beneficial bacterial populations warrant further consideration.

Simple, low-cost CCD camera system for active abrin detection. Abrin is a natural but lethal toxin produced and found in the seeds of the rosarypea (*Abrius precatorius* L.) The toxin has a similar mode of action to that of the select agent ricin, which is found in the seeds of the castor bean plant. There are many ways to test for the presence of abrin, but few assays can distinguish between the active (lethal) and inactive (nonlethal) form of the toxin. ARS researchers in Albany, CA, developed and built a simple, low-cost charge-coupled device (CCD) camera and applied it to cell-based assays. In presence of active abrin, the cells either produced a color change or a change in their fluorescent glow. The new fluorimetric method was able to detect as little as 0.1 pg/mL of active abrin. This simple and inexpensive method directly adds to the arsenal of tools for the accurate detection of abrin poisoning, which may occur for example, in cases of intentional food adulteration/contamination. (NP108, C1, PS3, Project No. 2030-42000-049-00D)

Bioinformatic tool for bacterial genome analysis. The development and implementation of next-generation sequencing has significantly improved our understanding of the bacterial genome architecture. However, there are still multiple bioinformatic hurdles to maneuver before a bacterial species becomes an informative annotated genome. Currently, bacterial bioinformatics generally entails using several stand-alone tools that make the process cumbersome and prone to specialist/human error. Developing technologies to eliminate these errors is a critical research need. ARS researchers in Athens, GA, and Colorado State University developed Reads2Resistome, a bioinformatic tool that streamlines this arduous effort. Reads2Resistome allows users with experience using basic Linux commands to analyze bacterial genomes using either short-read or long-read sequencing technologies. Reads2Resistome takes sequence reads as input and performs assembly, annotation, and genome characterization with the goal of producing an accurate and comprehensive description of the bacterial genome. Included in the analysis is determination and collection of all the antibiotic resistance and virulence genes, and other resistance elements within the main chromosome, or other elements such as plasmids or bacteriophage. The pipeline is executable on both Mac and Linux operating systems and is well suited for institutions and organizations that maintain or have access to a high-performance cluster for analyzing big data. Reads2Resistome is the first pipeline to our knowledge that performs both genome assembly and in-depth genome characterization. The new technology has been made publicly available on GitHub and is accessible to USDA researchers via SCINet.

Predicting *Salmonella* prevalence. *Salmonella* contamination of poultry and poultry products remains a critical concern for the USDA Food Safety Inspection Service. Farmers and inspectors alike need a way to predict the prevalence of *Salmonella* during the pastured poultry farm-to-fork continuum so that the correct and appropriate interventions can be applied. ARS researchers in Athens, GA, used random forest modeling, farm-management data obtained through questionnaires, and meteorological data to develop and validate algorithms that were effective at predicting *Salmonella* prevalence. The predictive modeling showed that years farming, broiler flock age, and dominant feed components were major farm management drivers of *Salmonella* prevalence in preharvest samples, whereas dominant feed components was the most relevant driver of *Salmonella* prevalence in postharvest samples. Average temperature, humidity, and high wind gust speeds prior to sampling were the meteorological variables that most closely correlated to *Salmonella* prevalence in preharvest samples. These data provide stakeholders with target variables to monitor to determine potential *Salmonella* food safety risks within their management systems.

Smartphone-based spectrometer. Industry and regulatory inspectors are required to monitor products for safety as they proceed through the food production and processing continuum. Normally samples are taken and sent to an internal or external laboratory for analysis using various types of assays. A major limitation of many food safety assays is that they require expensive equipment found only in centralized laboratories. Both industry and inspectors would prefer, where possible, that samples obtained in the field be analyzed on site, and data immediately be made available. ARS-funded scientists at the Center for Food Safety Engineering at Purdue University have developed a smartphone-based spectrometer that can resolve the visible range of spectrum in transmission mode and can be used to analyze many types of food safety assays. The overall cost of the spectrometer is only $200 and functions with an app that can visualize, record, and analyze the visible spectrum. The outcome is that this device could be incorporated into many types of assays with visual readouts to allow data to be used at the point the sample is taken, simplifying the assay process, and thus reducing the time required to obtain a result and transfer the data.