FY 2019 Annual Report for National Program 108 Food Safety

Executive Summary

Food Safety falls under Goal 4 of the Agency Strategic Plan**: Enhance Protection and Safety of the Nation's Agriculture and Food Supply**. 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 United States encompasses a diverse, extensive, and easily accessible system that is open to the introduction of pathogens (bacteria, viruses and parasites), bacterial toxins, fungal toxins (mycotoxins), 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, Agricultural Research Service (ARS) 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 ARS 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 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.

Relationship of this National Program to the ARS Strategic Plan: Outputs of NP108 research support the "Actionable Strategies" associated with the performance measures outlined below from the *USDA Strategic Objective 4.3:* Protect Public Health by Ensuring Food is Safe; *REE Goal 5:* Food Safety; and the ARS Strategic Plan for 2012-2017, *Strategic Goal 1.2* - Protect food from pathogens, toxins and chemical contamination during production, processing, and preparation.

> **Performance Measure 1.2.A:** Develop detection methodologies for foodborne pathogens and technologies for the rapid and sensitive detection of toxins, chemicals, and biologics that can be implemented for improved food safety and food defense. **Target 2017:** Cumulatively, five new technologies will be developed and transferred.
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> **Performance Measure** 1.2.B: Conduct and evaluate research that will lead to effective control and intervention strategies for the reduction of microbial, chemical, and other contaminants of the food supply, as well as elucidation of the molecular and physiological mechanisms that allow for persistence, survival, and transmission of foodborne pathogens in the populations and environment. **Target 2017:** Identify and evaluate potential control and intervention strategies for the reduction and control of foodborne pathogens and contaminants along the food production continuum.

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.

Selected Accomplishments for FY 2019

Escherichia coli O157:H7 transmission by cattle pest flies found in leafy greens. Leafy greens are a leading source of *E. coli* O157:H7 bacteria that cause human foodborne illness. Pest flies can carry this pathogen and may transmit it to leafy greens and other fresh produce. ARS scientists in Clay Center, Nebraska, determined the occurrence of *E. coli* O157:H7-positive flies in leafy greens planted up to 600 feet from a cattle feedlot, and assessed their potential risk for transmitting this pathogen to leafy greens. *E. coli* O157:H7 carriage rates of house, face, flesh, and blow flies were similar to each other and were greater than the carriage rate of stable flies. *E. coli* O157:H7 carriage rates were not different in flies found at different distances from the feedlot, ranging from 0 to 600 feet. Genetic subtyping showed that the majority of the *E. coli* O157:H7 found in the flies were of the same predominant subtypes found in the feedlot pen surface manure and the leafy greens, indicating the potential role for flies to transmit *E. coli* O157:H7 to the leafy greens. Due in part to this work and previous research, the produce industry has revised its guidelines for growers to increase the set-back distance between leafy greens fields and concentrated animal feeding operations. This information is critical for understanding the food safety risks associated with growing leafy greens in close proximity to cattle production, for determining safe distances between cattle feedlots and fresh produce that will reduce preharvest contamination and protect public health, and as potential guidance under the Produce Safety Rule as part of the Food and Drug Administration's Food Safety Modernization Act.

Development of hot-fill pasteurization of cucumber pickle spears as an alternative to tunnel pasteurization. For commercial production of acidified vegetable products, a tunnel pasteurizer is typically used for thermal processes. To help reduce energy costs and water use, ARS researchers in Raleigh, North Carolina, developed a hot-fill method for pasteurizing cucumber pickle spears in 24-ounce pickle jars. The method required refilling jars multiple times with a hot brine (around 175°F). The data showed that for cucumber spears a hot-fill method could achieve or exceed temperatures typically used for commercial pasteurization of pickle by most manufacturers. These conditions exceed published values needed for the required reduction of bacterial pathogens in acid and acidified vegetable products; they were also sufficient to meet Food and Drug Administration regulations and typical industry processing conditions needed for good quality texture and sensory properties. Although further development of processing equipment may be needed for inverting and refilling jars, the in-jar pasteurization process has potential application for cucumber spears and related products and may be used to save on the water usage and costs of currently used tunnel pasteurizers.

Location, season, and manure type affect pathogen survival in manure-amended soils. The Produce Safety Rule, part of the Food and Drug Administration's Food Safety Modernization Act, states that untreated manure must be applied 90 or 120 days prior to the harvest of edible produce crops to minimize contamination from pathogens that may be present in untreated manure. However, this interval was not scientifically validated. Over 12 separate field trials conducted in mid-Atlantic States over 4 years, ARS researchers in Beltsville, Maryland, and university collaborator showed that spatiotemporal factors (site, year, and season) affect

survival durations of *Escherichia coli* in manure-amended soils more than agricultural factors (manure type, organic or conventional management of soils, and depth of application) or weather effects. The results provide critical information to growers on the potential risk of produce contamination with specific raw animal manure application. The Food and Drug Administration will use these data to develop food safety standards for controlling bacterial contamination of fresh produce from soil. (NP108, C1, PSE, Project No. 8042-32420-006-00D)

Development of novel antibodies and detection assay to screen food samples for colistin-resistant bacteria. The recent discovery and rapid spread of the mobile colistin-resistant gene *MCR-1* in bacteria is undermining our ability to treat bacterial infections and threatening human health and safety. ARS researchers in Albany, California, developed novel polyclonal and monoclonal antibodies against *MCR-1* and *MCR-2*. An enzyme-linked immunosorbent assay using these antibodies was able to detect 0.01 ng/mL of *MCR-1* in buffer and 0.4 colony-forming units per gram of meat, including ground chicken, pork, and beef, demonstrating strong tolerance to complex food matrices. This immunoassay could be used for rapid and reliable screening of food samples contaminated with colistin-resistant bacteria, making this an important tool for reducing the risk of foodborne infections with antibiotic-resistant bacteria.

Neutralization of residual antimicrobial processing chemicals in broiler carcass rinse. The USDA's Food Safety and Inspection Service (FSIS) has established pathogen reduction performance standards for *Campylobacter,* a poultry associated-bacterial pathogen of significant concern both nationally and internationally, on broiler carcasses. Processors may apply antimicrobial processing aids as a spray or immersion to lower contamination on carcasses. In the United States, broiler carcasses are generally sampled by whole carcass rinse, and the potential exists for residual levels of antimicrobial processing aids to be carried over into the rinsate. It has been previously shown that, if unmitigated, such carryover can interfere with the detection of *Salmonella*. ARS researchers in Athens, Georgia, further demonstrated that unmitigated carryover of antimicrobial treatment can also interfere with the detection and recovery of *Campylobacter* in broiler carcass rinse samples. Traditional buffered peptone water was tested and found that it did not offer enough neutralizing capability to counteract residual antimicrobial activity of some post-chill processing aids (peroxyacetic acid, cetylpyridinium chloride, acidified sodium chloride, or a blend of acids) to allow full recovery of *Campylobacter*. A recently reported formulation for a neutralizing buffered peptone water, developed by ARS in Athens, Georgia, and currently being used by FSIS, outperformed the traditional carcass rinse medium and allowed significantly improved recovery of *Campylobacter* even in the presence of three of the four tested antimicrobial processing aids. Performance of the new carcass rinse medium with the fourth antimicrobial processing aid (acidified sodium chloride) was not different from the traditional formulation. Neutralizing buffered peptone water represents a significant improvement in the broiler carcass rinse method for detecting *Campylobacter*.

Determination of the minimum dose of sulfur (SO_2) dioxide fumigation to inactivate foodborne pathogens on table grapes. California produces 99 percent of the commercial table grapes in the United States, and fumigation with SO_2 at a single dose of 5,000 ppm-h is commonly used by the table grapes industry for field packaging of harvested product. The unit ppm-h is the

product of the concentration of SO_2 in parts per million multiplied by the duration of the fumigation in hours. ARS researchers in Albany, California, determined the minimum dose needed to inactivate foodborne pathogens inoculated onto freshly harvested table grapes under field packaging conditions. They found that the standard SO_2 dose was sufficient to kill all *Salmonella enterica* Thompson and *Escherichia coli* O157:H7 cells when each of the pathogens were inoculated at levels of 10,000 cells per grape; however, this dose did not inactivate the pathogens when they were at levels of 1,000,000 cells per grape. Similar results were obtained when the grapes were placed into cold storage after dosing with SO_2, indicating that the standard method was not sufficient to completely inactivate *S*. *enterica* Thompson and *E. coli* O157:H7 when higher levels of pathogens were on the grape surface. The results are important for the improvement of intervention strategies to ensure the safety of table grapes and were communicated to California growers through the California Table Grape Commission, who requested this research.

Development of imGLAD, a new pipeline for using metagnomics to detection pathogens. Rapid and accurate detection of pathogens from food samples is critically needed by the food industry, and the cost of whole genome sequencing from bacterial samples continues to decrease. ARS scientists in Albany, California, in collaboration with scientists at the Georgia Institute of Technology, developed a technique called imGLAD (in-silico-metagenomics for genome low-abundance detection) to detect human foodborne pathogens in samples of mixed DNA extracted from environmental samples. imGLAD was validated by detecting pathogenic *Escherichia coli* O157:H7 cells inoculated into field-grown, organic baby spinach leaves where the limit of detection was 100 cells/100 grams of spinach leaves. Metagenomics-based detection of pathogens is much faster than current culture-based methods and provides additional information that can be used for source-tracking foodborne outbreaks, which is essential information for public health investigations. This cutting-edge method is of interest to industries developing detection methods, growers, and public health agencies.

Cropland amendment with beef cattle manure minimally impacts antimicrobial resistance. There are concerns that using beef cattle manure to fertilize croplands increases the amount of bacteria with antimicrobial resistance (AMR) in these soils. If the increases persist until crops are planted, this could increase food animal and human exposures to AMR bacteria through feed and produce. ARS scientists in Clay Center, Nebraska, and colleagues treated one farm each in Nebraska, North Dakota, and South Dakota with either beef cattle manure, inorganic fertilizer, or no fertilizer as a control. Manure amendment did not increase AMR levels for 8 of the 10 AMR genes measured. For the other two AMR genes, AMR increases in croplands amended with manure occurred only at one location; the changes were transient and generally within the normal variation observed for control croplands. The scientists concluded the common practice of applying beef cattle manure to land in the Upper Midwest likely has extremely minimal impact on environmental AMR levels, feed safety, food safety, animal health, and human health.

Antimicrobial resistance is similar in food-service ground beef and pork regardless of antibiotic use claims. Antibiotic use during food animal production is theorized to significantly contribute

to antimicrobial resistance in humans. Beef and pork products produced from cattle and swine raised without antibiotics (RWA) in the United States are assumed to harbor lower levels of antibiotic resistance than conventionally (CONV) raised animals that may have received antibiotics. ARS scientists in Clay Center, Nebraska, found that CONV and RWA ground beef products contained microorganisms with similar levels of resistance to 13 different antimicrobials. Resistance to one antimicrobial was higher in CONV ground beef, whereas resistance to two antimicrobials was higher in RWA ground beef. For CONV and RWA pork chops, similar levels of resistance were found for all 16 antimicrobials examined. These results are consistent with prior research and provide further evidence that antimicrobial use in U.S. cattle and swine production does not significantly impact the antibiotic resistance present in beef and pork products.

In-feed chlortetracycline treatment in beef cattle does not impact antimicrobial resistance gene levels. The U.S. Food and Drug Administration recently implemented significant restrictions on the use of antimicrobials for growth enhancement in food animals. However, concern remains about the impact of in-feed antimicrobials on antimicrobial resistance. Chlortetracycline is an antimicrobial commonly fed to calves for 5 days shortly after entry into feedlots to prevent bovine respiratory disease. ARS scientists in Clay Center, Nebraska, found no differences in the levels of 10 antimicrobial resistance genes between chlortetracycline and control groups at any time from 5 to 117 days following a 5-day in-feed chlortetracycline regimen and concluded this treatment enhances animal welfare but does not increase antimicrobial resistance levels. (

Global and regional contributors to mycotoxin contamination of wheat and barley. *Fusarium* head blight (FHB) is a destructive disease of cereals crops worldwide and a major food safety concern because FHB pathogens can contaminate grain with vomitoxin and other fungal toxins (mycotoxins). FHB is caused by a diverse set of fungal species that make different mycotoxins. Understanding which FHB species and toxin types are present in an area is key to disease and mycotoxin control programs. ARS scientists in Peoria, Illinois, worked in collaboration with scientists in Uruguay and Brazil to identify and characterize FHB pathogens from their countries. The most common FHB pathogen of wheat and barley in Uruguay and Brazil, as well as the United States, is *Fusarium graminearum*, which can make a form of vomitoxin. However, a new species, *F. subtropicale*, was found in Brazil that produces a related mycotoxin with greater toxicity for humans and animals. Analyses of genetic diversity revealed that wheat and barley share a common FHB pathogen population that moves back and forth between these two hosts. The FHB pathogens in this study exhibited different levels of aggressiveness toward barley and different levels of resistance to two commonly used fungicides. These results provide new information on FHB pathogen and mycotoxin prevalence, host distributions, aggressiveness, and fungicide sensitivity that can be used to develop globally applicable and regionally targeted disease and mycotoxin control programs that improve crop production and food safety.

Detection of “masked” fungal toxins in corn. Fumonisins are a group of fungal toxins that are found worldwide in corn and other commodities. They cause a variety of diseases in domestic animals; in humans, they have been implicated in esophageal cancer of adults and neural tube defects of newborns. These mycotoxins are monitored to divert infested commodities from food and feed supplies. However, monitoring is complicated by the fact that fumonisins can form derivatives with other food components that can prevent their detection with commonly used screening techniques. These so-called “masked” toxins are an indeterminate hazard and rapid detection methods are needed. ARS scientists in Peoria, Illinois, developed a screening test that detected several of the masked forms of the fumonisin mycotoxins. Furthermore, to confirm the presence of such masked fumonisins in corn, a liquid chromatography high-resolution mass spectrometry (LC-HRMS) method was developed for their detection. The LC-HRMS method has facilitated the production of analytical standards, allowing confident identification of such toxins in corn. These tools will be useful for determining the extent to which the masked fumonisins contaminate corn and whether or not the masked forms represent a hazard to human or animal health.

Natural and environmentally friendly strategies to improve the postharvest food safety and shelf life of poultry products. The use of phytochemicals and probiotic bacteria as antimicrobials and biopreservatives in food products is safe, effective, and environmentally friendly. ARS scientists in Fayetteville, Arkansas, found that plant-based GRAS (generally regarded as safe) status compounds (e.g., carvacrol, caprylic acid, eugenol, beta-resorcylic acid, *trans*-cinnamaldehyde) and probiotic cultures from *Bacillus* and *Lactobacillus* sp. are very effective in reducing the presence of *Campylobacter* or *Salmonella* on poultry meat and eggs. They also found that edible coatings such as chitosan, gum Arabic, and pectin fortified with phytochemicals consistently reduced *C. jejuni* levels and modulated several genes that are critical for the survival and virulence of *C. jejuni*. This research has tremendous potential because *Campylobacter* is responsible for causing an estimated 1.3 million cases of foodborne illness in the United States. These plant phytochemicals can potentially provide conventional and organic poultry industries with economical and effective control strategies to control *Campylobacter* at various stages of poultry production and processing.

Reducing *Campylobacter* on poultry thighs using sequential treatments of antimicrobials. *Campylobacter* is a major concern for poultry processors as USDA performance standards have become stricter. ARS researchers in Athens, Georgia, evaluated the use of a low-pH processing aid and peracetic acid (PAA) applied in either individual- or consecutive-dip treatments to reduce *Campylobacter* in thighs. Combinations of dual low-pH dips, dual PAA dips, low pH then PAA, and PAA then low-pH were all evaluated against a control of dual buffer dips. PAA followed by low-pH dips showed significant reductions compared with all other treatments (99.9 percent compared with untreated). These data suggest that treatment with PAA, an oxidizing agent, following by an acidic low-pH treatment maximizes *Campylobacter* reduction. Treating with this sequence may allow processors to meet the strict performance standards on *Campylobacter* in broiler parts.

Using predictive algorithms throughout the pastured poultry farm-to-fork continuum effectively predicted *Listeria* spp. prevalence. To predict the prevalence of *Listeria* spp. during pastured poultry production, ARS researchers in Athens, Georgia, used random forest modeling of *Listeria* culture data in combination with questionnaire-based farm management data and meteorological data for the origin farms. The predictive modeling showed that time of year the flock was on pasture and the age of the broiler flock were major farm management drivers for *Listeria* prevalence in preharvest (feces, soil) samples, while brood feed and chlorination of the processing rinse water were the most relevant drivers of *Listeria* prevalence in postharvest (final product whole carcass rinse) samples. The meteorological variables that most closely correlated to *Listeria* prevalence in preharvest samples were average minimum temperature and average humidity over the 3 to 4 days prior to sampling. Further development and validation of these models will help pastured poultry farmers understand the variables that include the safety of their products, with the ultimate goal of providing them with management targets that can most easily be changed to reduce food safety issues within their flocks.
Genetic and environmental links between laying hens and *Salmonella enteritidis* contamination of eggs. Contaminated eggs produced by infected laying hens can transmit *S. enteritidis* to

consumers, but the influence of different poultry housing systems on the microbial safety of eggs produced by different genetic lines of chickens are not fully understood. ARS researchers in Athens, Georgia, assessed the production of eggs contaminated with *S. enteritidis* in groups of laying hens housed in conventional cages and colony units enriched with access to perches and nesting areas. Eggshell color is genetically determined; in the study, two groups of hens produced white eggs and two groups of hens produced brown eggs. All hens were experimentally infected with *S. enteritidis* and their eggs were collected for several weeks and tested for pathogen contamination of the internal contents. While *S. enteritidis* was found more often inside eggs from the two white egg lines than from the brown egg lines, housing type did not affect *S. enteridis* frequency. Hens producing one of the brown egg lines laid fewer contaminated eggs than any other line and the egg contamination frequencies of the two white lines differed significantly. These results demonstrate that *S. enteritidis* deposition inside eggs can vary between genetic lines of egg-laying hens, but different housing systems do not appear to influence these trends.

Using imagery from unmanned aerial vehicles (drones) for microbial water quality assessment in irrigation ponds. The microbial quality of water used for irrigation must be assessed to prevent the spread of microbes that can cause disease in humans. Microbial quality of irrigation water is evaluated based on concentrations of the indicator bacterium *Escherichia coli*. No recommendations have existed so far on where pond water samples should be taken for microbial analysis. ARS scientists from Beltsville, Maryland, proposed and tested a method of using drone-based imagery and artificial intelligence to obtain representative water samples for *E. coli* enumeration across irrigation ponds. Reflectance in different parts of the spectra are combined to characterize *E. coli* habitat in water. Results of this work provide the knowledge base for efficient microbial water quality sampling and indicate a new direction for monitoring microbial water quality, thus contributing to the improvements in food safety.

Absolute quantification of shiga toxin-producing *Escherichia coli* in beef with ddPCR. Harmful bacteria often possess a combination of distinguishing traits/markers that allow them to cause disease. Screening systems such as those used by the Food Safety and Inspection Service capitalize on the existence of these traits and can delineate potential disease-causing *E. coli* strains based on the presence of three such genetic markers. However, false positives result when a single sample contains more than one bacterium possessing one or two of the markers (but not all three of them) because the screening method does not define the specific organism from which each gene was derived. To overcome this shortfall, a new screening system known as droplet digital PCR (ddPCR) with the ability to determine when multiple genes are contained within a single source organism was developed and tested by the Food Science Division at Bio-Rad Laboratories, Inc. (Marnes-la-Coquette, France) in partnership with ARS researchers in Wyndmoor, Pennsylvania. Ultimately, this system saves costs by reducing staff hours and expenses associated with subsequent evaluation of false-positive samples and testing kits are expected to be released for purchase by Bio-Rad Laboratories, Inc. soon.

Gaseous chlorine dioxide (ClO_2) inactivates *Salmonella* while maintaining tomato. There have been a number of reports about the effectiveness of gaseous ClO_2 in inactivating various human pathogens associated with fresh produce. However, studies assessing microbial reduction,

impact on tomato quality, and impact on tomato nutrients are scarce. ARS scientists in Wyndmoor, Pennsylvania, determined the efficacy of gaseous ClO_2 in inactivating *Salmonella*, and its impact on sensory and nutritional quality of grape tomatoes. Results demonstrated that gaseous ClO_2 reduced populations of *Salmonella* on tomatoes by 99.99 percent and did not significantly affect appearance tomato texture, color, odor, or lycopene and ascorbic acid content. The study eases concern over damage of the fruit caused by gaseous ClO_2 and may help facilitate commercial applications of the gaseous antimicrobial on fresh produce.

Reduction of inorganic arsenic (iAs) concentration during cooking of rice. Rice is the staple food for half of the world's population, but it also contains a much higher concentration of inorganic arsenic (iAs), a Class 1 carcinogen, than other grains or vegetables. ARS researchers in Wyndmoor, Pennsylvania, developed an effective procedure to reduce iAs levels by first soaking one part rice at 80°C in 10 parts water, which is discarded after 10 minutes, and then cooking the rice in two parts water. On average, a 40 percent reduction in iAs concentration was achieved by this method due to the higher solubility of iAs in the hot water, which also contains the starchy gelatinous components from the rice. This presoaking method is easily implemented to reduce chronic arsenic exposure using common cooking practices. If this information is widely disseminated, those who follow this simple approach will cut their risk of cancer, which could affect billions of people worldwide.