FY 2018 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.

Component 1. Foodborne Contaminants

The production, processing, and distribution system for food in the United States is a diverse, extensive, and easily accessible system. This open system is vulnerable to introduction of contaminants through natural processes and global commerce, and by intentional means. Thus, the food supply must be protected from pathogens, toxins, and chemical contamination that cause disease or injury to humans. The ARS Food Safety Research Program seeks ways to assess and control potentially harmful food contaminants. ARS will conduct research and provide scientific information and technology to producers, manufacturers, regulatory agencies, and consumers to support their efforts to provide a secure, affordable, and safe supply of food, fiber, and industrial products.

Problem Statement 1. Population Systems

The goal of this research area is to identify and characterize the movement, structure, and dynamics of microbial populations within food-animal and plant systems, across the entire food continuum, from production through processing. At a microbial level, the diversity and complexity within environments and food matrices may alter the makeup of the populations, as well as cause change through spatial and temporal influences, or by the competitive or synergistic relationships among pathogens and commensals. Microbial populations can influence the safety of food, and the various environments in which they survive determine the success and impact of the microorganism. In turn, microorganism(s) may influence the conditions prevailing within the environment which also impacts their survival or ability to thrive. An example of identifiable area of study would include biofilms and the association of quorum sensing.

Components and emphasis for understanding and characterizing microbial populations and their environments must include epidemiology, ecology, and host-pathogen relationships. Epidemiologic studies of microbes within their environment, allows an analysis of the population therein. As such it enables the development of improved detection methods, provides a framework for integration of microbial genomic data with disease, and a mechanism to evaluate risk factors for microbial intervention and/or control. Ecologic studies determine the attributes and changes in various communities, that is, changes to populations in the same space. Such studies allow for a better understanding of the interactions and relationships, and the transmission and dissemination of pathogens and toxins in and among food producing animals and crops. Host-pathogen relationship studies provide an understanding of the acquisition of genetic traits, such as the development and movement of resistance genes; traits connected with colonization and evolution of virulence; and the role of commensals. Where appropriate, a metagenomics approach to selected research areas will be developed to determine the attributes of the ecological communities in which pathogens are found. Knowledge of the attributes, interactions and relationships within the community in which the pathogen lives is critical to the development of control and intervention strategies.

Within this Problem Statement it was critical to differentiate Food Safety from Animal Health. Certainly, there will be some overlap; however, this will be addressed at the Office of National Programs level. There will be an emphasis on how pathogens persist in animals and the related environment, and this will drive mitigation and prevention strategies, as well as guidelines, policy and regulation.

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. Systems Biology

Systems biology involves an integrated, multidisciplinary approach to study the complexities of biological components, a central problem to food safety. Identifying the components and players within the system allows the genetic components of bacterial, viral, and fungal pathogens and food-borne parasites, and their expression and products to be identified and directly related to the microorganisms. In order to study systems, quantitative technologies such as "omics" [genomics, proteomics, transcriptomics, metabolomics and metagenomics] combined with bioinformatics can be applied. There is an increased need for data gained from systems studies to be directly used for both pre- and postharvest food safety. For example, whole genome sequencing efforts using next generation sequencing (NGS) have increased and allowed regulatory agencies to identify and resolve outbreaks of foodborne illness (often for attribution purposes). It is recognized however, that the use of NGS requires extensive collaborations with other researchers.

The main goal of research developed in response to this Problem Statement is to utilize omic-technologies and apply them to the study of foodborne pathogens in complex food systems. For example, research will elucidate how microbes cause disease and assess their prevalence, pathogenicity (ability to infect and cause disease) and virulence (the severity of disease). Understanding pathogenicity and virulence is critical for intervention and control strategies, modeling, and providing data for the development of risk assessments by regulatory agencies. Pathogens have the capacity to readily and rapidly adapt and evolve, so pathogenicity and release of virulence factors is an issue at all stages of the food safety continuum. The prevalence and patterns of contamination in food sectors may vary considerably and needs to be assessed and evaluated carefully. Differences in microbial prevalence, pathogenicity and their virulence are observed across different food production and processing systems, at different sampling times, and by using various methods. Contamination patterns reveal variation in the pathogenicity and virulence and the presence of persistent or sporadic strains and evidence of bacterial transfer from production environments to processing, and from processing environments to food. Continual outbreaks of industry related bacterial contamination emphasize the continued need to examine pathogens in order to avoid public health risks.

Ongoing implemented microbial control strategies may lose their effectiveness, forcing the development of new production processes and products to maintain and improve the safety of foods. This in turn may restart the cycle of pathogen adaptation resulting from the changed environment and its stresses. Risk assessment(s) conducted by our regulatory stakeholders are also predicated on understanding the pathogen, the dose response, the behavior in foods, and any positive or negative influences that may affect virulence. Assessing the virulence of foodborne organisms and differences among serotypes/strains is critical in implementing new surveillance and intervention strategies. A critical issue within this Statement is the need to differentiate between microorganisms that are relevant to agriculture versus food safety and public health.

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. Microbial Contaminants: Technologies for Detection and Characterization

The challenge is the unequivocal detection and characterization of pathogenic microorganisms entering the food continuum (both pre- and postharvest). Detection and characterization are required at the earliest possible stage of the continuum to provide the necessary data for targeted interventions and reducing the need for recall of food products. Where possible, technologies must be developed that allow the most effective and rapid detection and characterization capabilities.

The focus of the research will be on the most promising technologies (depending on the matrix) or point of use, that is, whether the technology will be used for baseline studies, traceability and/or attribution forensics. This requires that decisions be made relative to what should be detected, and the required level of detection and characterization. It is noted that technologies that have the highest level of detection/characterization capability might not necessarily be the most practical, useful, economically viable, or easily implemented. High-through-put analysis is important, but it may be impractical. Promising technologies will be advanced through technology transfer, and where possible, and appropriate, will undergo validation through national or international bodies from academia, industry, and/or government sectors. Studies that suggest minimal outcome or impact will be terminated, and alternate approaches formulated.

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. Chemical and Biological Contaminants: Detection and Characterization Methodology, Toxicology, and Toxinology

Toxicology examines the relationship between dose and its effects on the exposed organism, whereas ***toxinology*** deals specifically with animal, plant, and microbial toxins produced by or accumulated in living organisms, their properties and their biological significance for the organisms involved. Both kinds of studies are required to reduce risks arising from contamination of food by chemical and biological contaminants.

The regulation and control of veterinary drugs, chemical residues, heavy metals, persistent organic pollutants, and biological toxins derived from bacteria, fungi and plants are an integral component of any food safety program. To protect public health and the environment, regulations have been passed and implemented that set limits on contaminants in edible agricultural products. Compliance and enforcement of these regulations is a critical role of the ARS National Program's stakeholders that requires the availability of practical detection and characterization methods for veterinary drugs (antibiotics, beta-agonists), chemical residues (dioxins, pesticides), heavy metals (As, Pb, Cd), and organic pollutants (polybrominated diphenyl ethers). In addition to regulatory monitoring, there is a need to understand the biological effects of any inadvertent contamination by humans or animals. In addition to toxicological and toxinological studies, this Problem Statement also includes research directed towards methods for detection and identification of mycotoxins, toxicity evaluation, and mechanism of action.

Accomplishments and promising technologies within this research area will be quickly advanced through technology transfer and where appropriate, will undergo validation through national or international bodies such as the Association of Official Agricultural Chemists (AOAC). These studies require multidisciplinary approaches to meet the challenge, and accomplishments may have far reaching effects regarding food biosecurity, regulations and trade issues.

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. Intervention and Control Strategies

Intervention and control strategies will assist in reducing or eliminating pathogens in food animals and their derived products, seafood, and plant crops during production and processing. Reduced shedding of zoonotic pathogens by food producing animals, and contamination of seafood and plant material will subsequently help reduce the pathogen load during slaughter/ harvesting and subsequent processing and storage. Some food processing/storage technologies have the ability to inactivate microorganisms to varying degrees; however, the intensities required can result in adverse functional and/or sensory properties, combined with a significant reduction in quality. Consequently, there remains a continued need to develop and subsequently combine new and/or innovative processing technologies. Interventions can be additive and/or synergistic, leading to improved control over pathogen growth without potential changes in food quality or reduction in nutrition. Research after an approved period that yields no outcome or requires the purchase of expensive equipment will be terminated, and alternate approaches formulated. If alternate approaches cannot be found, the project will be redirected to another priority. Unintended or unanticipated consequences of processing intervention strategies such as changes in virulence, production of toxins, pathogen resistance, selection of resistant strains, or changes in microbial ecology should be considered for further investigation.

The challenge is that the pathogen load on a product must be significantly reduced by any processing intervention strategy to avoid the consequences in food production resulting from "dirty in, dirty out" processing. There is also the concern that during processing the initial microbial load can be reduced but recontamination occurs with different strains or serotypes present or resident within the processing environment. Such concerns are valid because there are numerous observations that the pathogens present on product prior to processing are different from those found after processing. This variation in pathogen type has significant public health concerns since those pathogens initially found on the product may not be responsible for any foodborne outbreak and/or clinical outcome.

Research should also address, where possible, the integrated lethality for an intervention process. The purpose of the process lethality determination is to provide processors with science-based validation of the effectiveness of a specific process to destroy any microorganism of concern. For example, a thermal process needs to account for many variables including the initial pathogen load, multiple pathogens, pathogen strain variability, food structure, and the heating and cooling profile of the product. In-plant validation should be conducted to verify the intervention(s). The entire lethality process is incorporated into a systems approach to developing pathogen intervention or control strategies. Problem Statement 5 addresses a wide range of food products including animals, shellfish -seafood, and plant materials. The Problem Statement also includes biocontrol technologies for food crops contaminated by mycotoxins, such a tree nuts, corn and grains.

It is critically important within these studies that for development and validation of any process intervention a common or representative core set of pathogens or surrogates be used. This is critically important to make the intervention research results comparable both within and external to the Program. Core sets of strains for different pathogens will be made available through the ARS bacterial culture collection. If a specific strain is not available in the collection, ONP will facilitate researchers obtaining the appropriate isolate.

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. Predictive Microbiology/Modeling; Data Acquisition and Storage; Genomics Database

The tenet of predictive microbiology is that the behavior of any microorganism is deterministic and able to be, within limits, predicted from knowledge of the microorganism itself and the microorganism's immediate environment. However, it has been stressed by stakeholder groups that research should also include a greater emphasis on probabilistic modeling to balance the deterministic approaches. This would benefit predicting the behavior of pathogens under stressed conditions (more relevant to the food industry) where growth/inactivation is stochastic.

Behavioral predictions and models are accepted (globally) as an integral part of microbial risk assessment used to support food safety measures by both food safety regulatory bodies and industry. The Program does not develop or conduct Risk Assessments (RA), where RA is defined as the determination of a quantitative or qualitative value of risk related to a specific situation and a recognized hazard. However; the Program does conduct research and provide data when requested by our regulatory stakeholders (FSIS, FDA) for their use in conducting risk assessments.

The Program develops various modeling programs including; the Pathogen Modeling Program (PMP), a package of models that can be used to predict the growth and inactivation of foodborne bacteria, primarily pathogens, under various environmental conditions. In addition, the Predictive Microbiology Information Portal (PMIP) is geared to assist food companies (large and small) in the use of predictive models, the appropriate application of models, and proper model interpretation. The vision is that the PMIP will be the highway for the most comprehensive websites that bring together large and small food companies in contact with the information needed to aid in the production of the safest foods. The PMIP links users to numerous and diverse resources associated with models (PMP), databases (ComBase), regulatory requirements, and food safety principles.

All predictive models developed must be available for external examination, review and utility. If predictive models are developed for internationally accepted high priority pathogen-food combinations, then they could have a major impact for food companies in the USA and other countries producing and exporting food to the USA. This will require significant interactions with risk assessors and involvement in international initiatives such as National Advisory Committee on Microbial Criteria for Foods (NACMCF), Codex Alimentarius Commission (CODEX), Food and Agriculture Organization (FAO), and the World Health Organization (WHO). Collaborations with stakeholders must be strengthened with regards to what research needs to be conducted so as to effectively utilize the inherent ARS expertise and modeling systems mechanisms.

Data acquisition and storage: ARS and international institutes, including Institute Food Research (IFR-UK) and the University of Tasmania (UTas), as well as associate members University of Querétaro, Mexico; Unilever Research, UK; Agricultural University of Athens, Greece; National Food Research Institute, Japan; Hokkaido University, Japan; and Rutgers University also developed and maintains a publically available global food safety database**,** ComBase **-** *a Combined dataBase for predictive microbiology* – which is the number-one web-based resource for quantitative and predictive food microbiology in the world. Its main components include a database of observed microbial responses to a variety of food-related environments and a collection of relevant predictive models. The purpose and goal of ComBase is to provide an electronic repository for food microbiology observations and to make such data and the generated predictive tools freely available and accessible to the entire food safety community. Data acquisition and use is an interdisciplinary research challenge that translates into safer products and improved public health.

Genomics as a functional and critical part of omic-technologies holds great promise for improving the early detection, prevention and control of current and emerging foodborne pathogens, thus contributing to improved food safety and consequently public health. Genomics have the potential as a partner or replacement of culture-based techniques. Food safety regulatory agencies, USDA and the FDA, have discussed and are planning to implement the increased use of genomics, in particular partial and/or whole genome sequencing (P/WGS) for both regulatory monitoring, attribution and potentially for revising risk assessments. Implementation of such a redirection requires developing a coordinated system of genomic sequencing technology for routine testing. Critical within this issue is the development of an ARS database from our national and international sequencing/annotation efforts. For this work, a common or representative core set of bacterial pathogens or surrogates will be available. Additional data from isolates studies obtained from national and international collaborations will be incorporated. Allied to the sequencing efforts will be meta-data descriptors. This research will be part of a larger international initiative, the Global Microbial Identifier (GMI), a global, visionary taskforce including more than 30 countries who share an aim of making novel genomic technologies and informatics tools available for improved global infectious disease diagnostics, surveillance and research, by developing needs and end-user based data exchange and analysis tools for characterization of all microbial organisms and microbial communities.

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. Antimicrobial Resistance

The discovery of antibiotics transformed human and veterinary medicine and saved millions of lives in the United States and around the world. The rise/increase of antibiotic-resistant bacteria represents a serious threat to both animal and human health and the economy. The concern for the development of antimicrobial resistant (AMR) bacteria has resulted in the development of both national and international strategies to address the issue. In 2014, the President signed an Executive Order, and a strategy was developed by multiple agencies to begin addressing AMR at the National level. Even though the USDA is not the lead regulatory agency for antibiotic use and AMR, USDA-ARS is an important part of the solution.

Areas of concern include detecting, measuring, and assessing the amount of AMR bacteria within the production animal populations with an emphasis on foodborne pathogens. In addition, developing alternative strategies to minimize the use of antibiotics in production animals while maintaining and improving animal health and ensuring safe food for consumers is a critical need.

Currently, the role of feeding antibiotics to production animals on the development of AMR bacteria and the impact on public health is not well defined. In addition, there is a critical need for the development of alternative strategies to reduce the level of antibiotic use as well as developing mitigation strategies for foodborne AMR bacteria in food producing animals. These areas are cross-linked with Problem Statements 1 and 2 within the Action Plan

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.

Selected Accomplishments for FY2018

An effective method to dry and decontaminate wet whole almonds. California produces 80 percent of the world's almonds with a value of more than $5.33 billion. Contamination of almonds with *Salmonella* has caused several large and expensive recalls by the industry and outbreaks of human illness. The occurrence of rain during the harvest season may result in the complete loss of an almond crop due to increased risk of microbial contamination and lack of adequate drying technology. ARS scientists in Albany, California, developed an effective and energy-saving new technology based on sequential infrared heat and hot air to simultaneously dry and decontaminate wet whole almonds. The results were provided to industry and contributed to ARS receiving the 2018 Research and Development Award by the Institute of Food Technologists.

Improvements in radio frequency pasteurization of shell eggs. Raw shell eggs can be contaminated with *Salmonella*, causing illnesses and recalls. ARS developed and patented a radio frequency pasteurization (RFP) process that produced safer eggs with exceptional quality in a small-scale prototype. Now ARS researchers in Wyndmoor, Pennsylvania, in collaboration with a CRADA industry partner, assembled and successfully tested a larger-scale RFP unit, thus paving the way for a commercial-scale RFP unit. In addition, two breakthroughs were achieved that will facilitate commercialization. The first was the modification of RFP to operate at 40.68 MHz, which is an international frequency reserved for industrial, scientific, and medical purposes. The second modification of the RFP reduces the cost to use this technology on eggs. This will save between $10,000 to $100,000 per RFP unit. This technology can address a significant, widespread source of foodborne illness and make shell eggs safer.

Recognition of emerging food-pathogens using artificial intelligence. Pathogen detection and data analysis are often limited to the types of samples present in a database. Problems often occur when new bacteria not present in the database are encountered. ARS in collaboration with the Center for Food Safety Engineering at Purdue University in West Lafayette, Indiana, explored the application of an artificial intelligence (AI) system to phenotypic characteristics of various foodborne pathogens. The aim was to determine the ability of the AI to identify the number of present pathogenic classes, and to recognize new, unknown classes of foodborne pathogens that were not present in the databases. The research developed a functional prototype of an emerging pathogen detection system using AI methodology primarily based on the pattern-recognition neural network created by data scientists at Google initially for the goal of classifying natural images. The technology integrates the cutting-edge machine-learning tools with a unique optical phenotypic biosensing device developed in collaboration with Purdue University. The result demonstrated the tremendous potential of the AI technology in the areas of biosurveillance, biothreat detection, and agricultural biosafety. Additionally, it emphasized that leveraging the existing state-of-the-art informatics tools employed by the leading U.S. data management companies will lower the cost of adoption of the new AI technologies by food producers and regulatory agencies.

Raman sensing technology for chemical hazard detection. Detection of chemical contaminants during commercial food processing is a critical issue for rapid authentication of food ingredients and to determine potential adulteration. ARS scientists in Beltsville, Maryland, developed a linescan high-throughput Raman imaging method and apparatus for rapid, nondestructive detection of chemical contaminants in food materials. The system can directly and rapidly analyze a sample powder in only 10 minutes, compared with conventional instruments that might take hours to perform the same analysis. The system has imaged a variety of food powders mixed with chemical additives, and results indicate that the system can provide quantitative measurement of chemical adulterants. This technology (patent No. 9,927,364) provides a practical industrial screening tool to address chemical contamination and adulteration of food products.

Development of sensitive detection assays for abrin toxin. Abrin is a natural toxin found in the seeds of the jequirity pea. The toxin is similar to ricin, a poison found in the seeds of the castor oil plant. Abrin, like ricin, is considered a select agent toxin and a potential bioterror weapon. Researchers in Albany, California, developed new monoclonal antibodies against abrin and assembled sandwich enzyme-linked immunosorbent assays (ELISA) (similar to a pregnancy test) capable of detecting a mixture of abrin isoforms. The ELISA can detect as little as 1 nanogram/milliliter of abrin in phosphate-buffered saline, nonfat milk, and whole milk, an amount that is significantly below concentrations that would pose a health concern for consumers. Fortuitously, some of these antibodies can also neutralize abrin toxicity in cell-based assays, so they may have vaccine potential. Easy, cost-effective, and more rapid methods of detection for abrin toxins are critically necessary during incidences of deliberate or suspected food contamination.

A box liner with a slow-release sulfur dioxide pad enhances the killing of foodborne pathogens. California produces 99 percent of the commercial table grapes in the United States. Ensuring that they arrive safely and not contaminated at food stores for consumer purchase is a critical issue for the California Table Grape Commission and California agriculture. ARS researchers in Albany, California, at the request of the Commission, examined the survival of three common but important foodborne pathogens: *Listeria monocytogenes*, *Escherichia coli* O157:H7, and *Salmonella enterica* Thompson, inoculated on commercially packed table grapes under simulated refrigerated transit conditions. Results showed that a box liner in the shipping container enhances the bactericidal effect of a sulfur dioxide (SO_2) pad in a pathogen-dependent manner. The use of slow release SO_2-generating pad combined with box liner was found to be effective in killing *L. monocytogenes* and *S. enterica* Thompson, whereas the use of a SO_2-generating pad alone was more effective in killing *E. coli* O157:H7.

Effect of raising beef cattle without antibiotics on the occurrences of antimicrobial resistance. There is a significant societal concern that traditional antimicrobial use patterns for food-animal production have contributed to the occurrence of antimicrobial resistance (AMR) in human infections. In response to this concern, ARS researchers in Clay Center, Nebraska, compared fecal AMR levels between U.S. beef cattle produced conventionally, with no restrictions on antibiotic use other than regulatory compliance, and U.S. beef cattle raised without antibiotics. Fifty of 67 individual microbial AMR levels were not different between production systems, whereas 17 of 67 levels exhibited significant increases in conventional animals. However, although these increases in AMR were statistically significant, they were so small they are not likely biologically significant. More importantly, cattle raised without antibiotics typically grow slower, so they must be fed 50 days longer and thus produce about 2,500 pounds more manure.

Therefore, the 31 percent increase in amount of manure from cattle raised without antibiotics more than offsets the small reduction in a few resistances and may actually increase the total AMR in the environment. Thus, beef cattle production without any antibiotics would not be expected to reduce the amount of AMR contributed to the environment compared with conventional production.

Invasion of internal organs by *Salmonella* ser. Enteritidis (*S.* Enteritidis). The transmission of *S.* Enteritidis inside contaminated eggs laid by infected hens is a leading human health concern and, in 2010, an outbreak of egg-associated salmonellosis cost the industry more than $100 million. It isn't clear how proposed changes to poultry housing systems affect the microbial safety of eggs produced by different genetic lines of chickens. ARS researchers in Athens, Georgia, assessed how the internal organs of four commercial genetic chicken lines (two brown egg and two white egg lines) housed in both conventional cages and colony units enriched with access to perches and nesting areas were affected by *S.* Enteritidis infection. The researchers recovered *S.* Enteritidis from intestinal samples of the white egg lines at a significantly higher frequency than from the brown egg lines in both conventional cage and enriched colony housing systems. The frequency of intestinal *S.* Enteritidis recovery was greater in one line of brown egg-laying hens raised in conventional cages than in enriched colonies. Selecting genetic stocks of laying hens with low susceptibility to *Salmonella* infections under new housing conditions may contribute to risk reduction in egg production.

Genetic features unique to supershed *Escherichia coli* O157. Shiga toxin-producing *E. coli* serotype O157 causes intestinal disease in humans, and although it colonizes cattle intestines, it does not cause disease. Cattle that shed O157 in ≥10,000 colony forming units per gram of feces are referred to as supershedders, and limiting shedding from cattle is important for maintaining a safe and secure food supply. Supershedding cattle can easily disseminate O157 within large herds, which may increase transmission to meat at slaughter. The supershed O157 bacteria may possess unique genetic features that contribute to their increased adherence and persistence in the bovine intestinal tract. In a recently completed study by ARS researchers in Ames, Iowa, the genomes of two supershed O157 strains of *E. coli* were sequenced, analyzed, and compared with other previously characterized O157 strains. Supershed O157 have unique, recently acquired genetic features, especially in genes associated with motility, adherence, and metabolism that could influence their persistent colonization of bovine intestines. The proteins encoded by these genes could be developed into blocking therapies aimed at preventing supershed O157 colonization of cattle.

ComBase, an international microbial modeling database. ARS with the University of Tasmania (UTas) continues to develop ComBase is an online tool for industry and regulatory agencies to use in describing and predicting how microorganisms survive and grow under a variety of food-related conditions. ComBase further assists users in predicting and improving the microbiological safety of foods and assessing microbiological risk in foods. One of ComBase's principal features is the database, which gives users access to more than 70,000 data entries. New enhancements to ComBase include the following: each data record now indicates the number of times it has been viewed and downloaded; a YouTube channel and tutorials are available; a private data section with ComBase is available to embargo data until a publication has been released; ComBase has social media accounts on Facebook, LinkedIn, and Twitter; a new search feature has been added to the browser; each record now indicates the date that the record was added to ComBase; and an improved and simpler data donation template, plus instructional videos, have been added to the Data Submission page. The ComBase was exhibited at European and U.S. conferences to increase interactions with users and data donors.

Detecting the "masked" derivative of T-2 toxin in wheat. Mycotoxins are secondary metabolites produced by fungi such as *Aspergillus* and *Pennicilium*. Many mycotoxins are highly toxic to humans and animals, causing a variety of secondary conditions, including cancer. T-2 toxin is produced by *Fusarium* fungi that infest oats, wheat, barley, rye, maize, and rice. Plants protect themselves from T-2 toxin by metabolizing it and adding a sugar component; this results in the formation of the metabolite T2-glucoside, which is not detected by routine tests for T-2 toxin. Digestion of the T2-glucoside in the human or animal gut may lead to the regeneration of T-2 and its associated toxicity. A novel method for detecting both toxin forms was developed by ARS scientists in Peoria, Illinois, using a new technology, called imaging surface plasmon resonance (iSPR). The method was able to detect very low levels (4 ppb) of both forms and was validated for meeting the criteria for testing grain exports at a level established by the European Union (100 ppb). Development and validation of the method ensures both the toxin and its metabolite are detected at levels that ensure food safety and support the export of U.S. commodities.

Detection of drug residues. Zilpaterol is an additive that enhances the efficiency of feed conversion and causes cattle to increase in size. The compound has been approved for cattle production in North America, Central America, and South Africa, however, its use is strictly banned by the European Union, China, and many other countries since residues may affect humans. Because zilpaterol use is legal in only a few countries, the rapid and sensitive detection of it in live animals or in animal tissues is of critical concern to regulatory and trade officials worldwide. ARS scientists in Fargo, North Dakota, optimized two mass-spectrometry based techniques to allow the rapid, sensitive, and semi-quantitative screening of zilpaterol residues in animal urine and tissues in about 30 seconds per sample with little to no sample preparation needed. The new instrument-based techniques have the potential to be adapted for field use, and to be used for the rapid detection of numerous other chemical residues of importance to animal agriculture.

Reduction of inorganic arsenic content in cooked rice. Rice is a staple food for half the world population, but it may contain higher levels of toxic inorganic arsenic (iAs) than other common crops. Chronic exposure to iAs through rice is a worldwide concern for consumers. ARS researchers in Wyndmoor, Pennsylvania, developed an effective protocol to reduce iAs in cooked rice by presoaking the rice in hot water for 10 minutes, then discarding the water before cooking and using a rice cooker until the rice becomes dry. Previous efforts to reduce iAs in cooked rice took longer and were less effective. This more rapid protocol achieved similar or better iAs reduction by raising soaking temperature above the gelatinization temperature of rice starch, which causes higher diffusion kinetics to reduce iAs levels. Implementation of this protocol in daily practice would cut the cancer risk for rice consumers and improve their long-term health prospects.

Analysis of pesticides and environmental contaminants in meats and poultry. The USDA Food Safety and Inspection Service (FSIS) is responsible for routine monitoring of pesticide residues in meat and poultry products to assure that regulatory tolerances are not exceeded, and foods are safe for human consumption. ARS scientists in Wyndmoor, Pennsylvania, developed and validated a fast, efficient, high-throughput analytical method for FSIS-priority pesticides and environmental contaminants in meat (swine, cattle) and poultry (chicken) muscle. The high throughput method for simultaneous determination of pesticides and environmental contaminants is conducted using QuEChERS-based sample preparation, extraction, and cleanup. The method was validated at three spiking levels at and below U.S. regulatory levels of concern, and satisfactory recoveries (70–120 percent) and relative standard deviations ≤20 percent were demonstrated for 219 analytes. The method was transferred to FSIS laboratories and is currently being implemented for routine monitoring of contaminants. Implementation of the new method is expected to improve regulatory monitoring, reduce the cost of sample analysis, increase sample throughput, and provide reliable data for more contaminants of concern.

Effect of atmospheric carbon dioxide levels on *Aspergillus flavus*. The impact of temperature and water on the ability of the fungus *A. flavus* to be geographically distributed, grow, infect crops, and produce aflatoxin (a potent cancer-causing compound) has been well characterized. However, the effects of these two environmental factors under higher CO_2 conditions have only recently been characterized. ARS researchers in New Orleans, Louisiana, demonstrated that increased atmospheric CO_2 levels can lead to an increase in aflatoxin production. The expression patterns of genes present in several secondary metabolites (compounds that are often toxic and can also be involved in fungal development, survival, and infectivity) and gene clusters, including the aflatoxin cluster, were modified after exposure to higher CO_2 levels. Several gene networks that control fungal biological processes such as DNA replication, amino acid synthesis, and production of conidia (asexual reproductive structures also known as spores) were also affected. These results demonstrate the effect that elevated CO_2 levels can have on important fungal biological processes. These data are being used by modelers for predicting the levels of toxin contamination under various environmental conditions. These models are providing insight on how remediation efforts will be influenced by future global environmental conditions.

Surface pH of fresh beef as a parameter to validate effectiveness of lactic acid treatment. The food safety system implemented by beef processors includes the use of antimicrobials such as lactic acid sprayed on beef carcasses to mitigate bacterial contamination. Antimicrobial interventions used by beef processors must be validated under actual processing conditions. But antimicrobial intervention sprays are applied in various concentrations and under various spray volumes and pressures, making validation studies for commercial beef processors cumbersome and expensive. ARS researchers in Clay Center, Nebraska, conducted a study to determine whether the surface pH of a beef carcass after applying lactic acid could be used as an effective and inexpensive measurement of antimicrobial efficacy and to reduce pathogenic bacteria if present. Results indicated that carcass surface pH was very effective in validating reductions in both *E. coli* O157:H7 and *Salmonella* on beef carcasses. Surface pH measurement can now be used by the beef processing industry to efficiently validate the effectiveness of using lactic acid in pathogen reduction efforts in beef, thereby improving food safety and reducing costs to beef processors.

A new natural antibiotic alternative for swine. Livestock farmers are under increasing pressure to reduce their use of antibiotics to control disease during production and need new technologies to help them maintain the optimal health and well-being of their animals. ARS scientists in College Station, Texas, in collaboration with scientists at the Norman E. Borlaug Institute for International Agriculture, determined the efficacy of the *Nigella sativa* (black cumin) plant, as a potential substitute for conventional antibiotics currently used in swine production. The work established that feeding black cumin to pigs dramatically improved the animals' growth efficiency and helped resist colonization by *Escherichia coli*, which is particularly pathogenic for young pigs. These results provide important information on a potential new feed additive that, when combined with other feed ingredients and good management, can help pig farmers improve the health and well-being of their young animals. Ultimately, these results will help pig farmers find new ways to safely and economically produce high-quality and wholesome pork products at less cost.

Fungi responsible for fumonisin mycotoxin contamination in corn. Fumonisins are among the mycotoxins of greatest concern to food and feed safety because they are frequent contaminants in corn, have potential to cause esophageal cancer in adults and neural tube defects in newborns, and can cause multiple diseases in some domestic animals. Although the fungus *Fusarium verticillioides* has been considered the primary cause of fumonisin contamination in corn for decades, the recent finding that another corn-associated fungus, *Aspergillus niger*, can produce fumonisins has raised concerns that it too is responsible for fumonisin contamination. In a multiyear collaboration with scientists at Iowa State University, ARS scientists in Peoria, Illinois, demonstrated that infection of corn ears with *A. niger* did not result in accumulation of significant levels of fumonisins. This finding indicates that *A. niger* does not contribute significantly to fumonisin contamination in corn, and that work by industry, academia, and research agencies to prevent fumonisin contamination in this important crop should focus their efforts on *F. verticillioides*.

Resistome analysis of dairy calves and lactating dairy cows. The role of dairy cattle in the transmission of antibiotic-resistant bacteria (ARB) to humans is unclear. However, dairy cattle are reservoirs of ARB, and dairy calves harbor more ARB than adult cows. ARS scientists in Beltsville, Maryland, sequenced the genomes of the bacteria (metagenome) found in the feces of preweaned dairy calves and lactating dairy cows from 12 farms. Results indicated that feces of preweaned dairy calves have a significantly different bacterial community population than lactating cows. Similarly, the resistomes (the totality of antibiotic resistance genes in the bacteria) were significantly different between the adults and calves. The relative abundance of antibiotic resistance genes was highest in feces from the younger animals. Results of this analysis support the observation that dairy animals are colonized with ARB at a very young age and indicates that more information is needed to determine the factors that affect this early colonization to determine mitigation efforts that dairy operations could use to potentially reduce the abundance of antibiotic resistance in these animals.

Microbiological quality of spinach. Water scarcity is a serious issue for fresh produce production, and alternative water sources such as reclaimed (reused) wastewater and roof-harvest water may help overcome water scarcity while maintaining food security and food safety. ARS scientists in Beltsville, Maryland, grew spinach in a controlled environment chamber and was irrigated with alternative water for 4 weeks, and then spinach samples were collected weekly and analyzed for bacterial populations. A single irrigation with alternative water containing higher populations of total and fecal coliform bacteria did not necessarily result in higher populations of the coliform bacteria on spinach leaves; however, repeated irrigation with reclaimed wastewater resulted in higher numbers of *E. coli*–positive spinach samples. Pathogens were not detected from any water or spinach samples. Roof-harvest water had higher microbial quality than the reclaimed wastewater. Roof-harvest water irrigation did not increase the populations of fecal bacterial indicators on the irrigated spinach plants. These results show the potential use of alternative water sources for irrigation without affecting the microbiological safety and quality of produce. This information is critical for industry and regulatory agencies under the umbrella of the produce section of the Food Safety Modernization Act.