FY 2017 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 emphasizes 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.

<u>Anticipated Products</u>

- 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 in order 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 FY2017

The U.S. Environmental Protection Agency registers biopesticides for aflatoxin management. Biopesticides based on atoxigenic strains of *Aspergillus flavus* have become the most widely used intervention for preventing aflatoxin contamination. However, these biopesticides must be approved by and registered with regulatory authorities, and each target crop and each atoxigenic genotype require additional regulatory action. Thousands of atoxigenic genotypes of *A. flavus* exist with broad adaptation, but regulatory approval for use in commercial products has only been granted for a few genotypes. ARS researchers in Tucson, Arizona, addressed this through direct interactions with regulatory authorities, field and laboratory experimentation, and collaborations with the Arizona Cotton Research and Protection Council, the University of California, the International Institute of Tropical Agriculture, commodity groups, and several national governments. The result is new and expanded registrations of biopesticides for preventing aflatoxin contamination, including approval for new target crops (figs and almonds), additional *A. flavus* genotypes, and less restrictive handling requirements. Full registrations for the United States, Senegal, and Burkina Faso were added to existing registrations in the United States (partial), Kenya, and Nigeria.

New natural fumigant that inhibits pathogenic fungal. ARS researchers at Albany, California, identified benzaldehyde-1 (BA-1) as a natural fumigant that can effectively prevent fungal growth in tree nuts. Co-application of BA-1 with a conventional fungicide fludioxonil (FD) inhibited growth of FD resistant strains of Aspergillus sp. and Penicillium sp. Benzaldehydes prevented fungal growth by disrupting metal chelation in the metabolism processes. BA-1 also inhibited the germination of weed seeds, a natural reservoir for fungal pathogens. Thus, natural products such as BA-1, when used alone or in combination with existing fungicides, could serve as potent antifungals by controlling the growth of pathogenic fungi and their reservoirs. This research benefits crop industries by identifying safe and natural antifungals that can replace or reduce the use of existing fungicides.

Development of a host plant volatile blend that attracts navel orangeworm in almonds. Damage of fruit nuts by navel orangeworm has been associated with increases in *Aspergillus* infection, a risk factor for aflatoxin contamination. ARS researchers in Albany, California, developed and patented (U.S. Patent No. 9,655,366) a new blend of host plant volatiles that attract the navel orangeworm to almonds. The efficacy of this blend in attracting both male and female navel orangeworms was demonstrated in orchards and used in the monitoring of worm infestation and as an aid in pest management. The navel orangeworm blend is being developed as a product for commercialization. This new technology will help farmers monitor and reduce navel orangeworm infestation in orchards and, thus, eliminate aflatoxin contamination.

Probiotic bacteria that inhibit botulinum neurotoxin intestinal cell binding. Probiotic microorganisms have been extensively studied for their beneficial effects in protection from allergens, pathogens, and toxins. ARS researchers in Albany, California, evaluated the effect of probiotic bacteria on the intestinal binding and absorption of botulinum neurotoxin (BoNT) serotype A. BoNTs are some of the most poisonous natural toxins known and are threats to public health and safety. Several probiotics that were tested (*Saccharomyces boulardii*, *Lactobacillus acidophilus*, *L. rhamnosus* LGG, and *L. reuteri*) blocked BoNT/A intestinal uptake in a dose-dependent manner, whereas a non-probiotic strain of *Escherichia coli* did not. These results show, for the first time, that probiotic treatment can inhibit BoNT/A binding and internalization in intestinal cells and may lead to the development of new therapies for treating BoNT poisoning.

Monensin and fecal shedding of E. coli O157:H7 in cattle. Monensin is fed to cattle to improve animal growth and feed efficiency, and to reduce methane production from the rumen. Previous research has reported conflicting results regarding the effects of feeding monensin to cattle on E. coli O157:H7 fecal shedding. ARS researchers at Clay Center, Nebraska, conducted a series of studies over 2 years to evaluate the potential effects of feeding different levels of monensin on fecal shedding of E. coli O157:H7. Dietary monensin did not appear to impact fecal presence or absence for E. coli O157:H7 in the studies. However, feeding monensin at the highest allowable level increased the percentage of animals that were shedding E. coli O157:H7 at high levels. This research indicates that feeding ionophores like monensin is not inhibitory to E. coli O157:H7 in the bovine gut, but at high levels may alter colonization and shedding in cattle.

Antimicrobial resistance in meats from animals produced with and without antibiotics. Meats produced from animals raised without antibiotics (RWA) are perceived to harbor lower levels of antimicrobial resistance than meats from animals produced with no restrictions on antimicrobial use (conventional). ARS researchers in Clay Center, Nebraska, measured the prevalence and concentrations of antimicrobial resistant bacteria and the levels of 10 antimicrobial resistance genes in ground beef and pork chops from animals raised without antibiotics or by conventional methods. The researchers found that generally the levels of antimicrobial resistance were similar between the meats from animals raised either way. These results demonstrate that conventional beef and pork products do not pose a greater risk of exposure to antimicrobial resistance than RWA products.

Characterization of pathogenic E. coli on veal hides and carcasses. Beginning in 2012 the USDA FSIS increased scrutiny of bob veal (calves less than 3 weeks old) and formula-fed veal (calves 20 weeks in age) when a higher percentage of positive tests for pathogenic Shiga toxin-producing Escherichia coli (STEC) were found in veal compared to beef. To investigate this problem, ARS researchers in Clay Center, Nebraska, measured the levels and prevalence of E. coli O157:H7 and non-O157 STEC on veal hides and carcasses just after the hide was removed and before any antimicrobial interventions were applied at five veal processors. A year later follow-up samples were collected at three of the processors. Significantly more non-O157 STEC were found on veal hides and carcasses than E. coli O157:H7, as compared to beef where the opposite has been reported. The follow-up samples showed that processing had improved and less STEC was detected on carcasses. In addition, a greater proportion of bob veal was found to be contaminated by STEC compared to formula-fed veal. Changes in processing have improved the safety of veal.

Antimicrobial resistance in mature beef cows is not correlated to antimicrobial use. There is a growing concern that antimicrobial use in food animals is leading to increased antimicrobial resistance. Previous studies have shown that antimicrobial treatment of feedlot cattle results in an increase in an antimicrobial-resistant bacterial population that returns to pretreatment levels approximately 14 to 36 days after treatment. Because beef cows are long-lived, they are more likely to receive antimicrobial treatments than feedlot cattle, albeit spread over a longer period of time. ARS researchers in Clay Center, Nebraska, compared the occurrences of resistance to antimicrobials in bacteria from beef cows for which complete antimicrobial treatment records were available. Approximately half of the cows sampled for this study were treated with antimicrobials for the treatment of disease, whereas the other half did not receive any antimicrobial treatments over their lifetime. The studies found that the occurrence of antimicrobial-resistant bacteria was not associated with or predicated on prior history of antimicrobial treatments or duration of time since the last antimicrobial treatment. Other as yet unknown factors more strongly influenced the observed levels of antimicrobial-resistant bacteria in beef cows.

Treatment of cattle hides with a bacteriophage before processing. *Escherichia coli* O157:H7 is a major food safety concern for the beef industry. Several studies have provided evidence that cattle hides are the main source of beef carcass contamination during processing and that reductions in the *E. coli* O157:H7 load on the hides of cattle entering processing facilities will lead to reductions in carcass contamination. Bacteriophage, viruses capable of killing bacteria, have been proposed as a novel technology to reduce the levels of *E. coli* O157:H7 on cattle hides. ARS researchers in Clay Center, Nebraska, evaluated a commercialized bacteriophage spray applied to cattle before they entered a processing plant for the spray's ability to reduce *E. coli* O157:H7 contamination of cattle hides and carcasses. The results demonstrated that treatment did not produce a significant reduction in *E. coli* O157:H7 on hides or beef carcasses during processing, and thus treatments using bacteriophage before processing may not improve beef safety.

Rapid residue screening test developed. Zilpaterol is an FDA-approved beta-agonist feed additive that increases feed efficiency, improves growth rate, and produces lean meat in livestock. Several major U.S. trading partners do not allow the import of meat from animals fed zilpaterol and in the United States zilpaterol is illegal to use in some food animal species. ARS researchers in Fargo, North Dakota, developed a sensitive, selective, inexpensive, and rapid test to determine whether animals have been exposed to zilpaterol. The assay, which is similar to an over-the-counter pregnancy test, can be used on-site with minimal training, and results are ready in about 10 minutes. The accuracy and sensitivity of the assay was verified in tissues and urine from animals exposed to zilpaterol. This simple and inexpensive assay could be used to determine accidental, illegal, or purposeful zilpaterol exposure.

Incidence of *Salmonella enterica* in swine. *Salmonella enterica* is an important cause of foodborne illness throughout the world. ARS researchers in College Station, Texas, in collaboration with colleagues at Texas Tech University and Texas A&M University, hypothesized that *S. enterica* in cattle lymph nodes could pose a risk for human infection if these lymph nodes were included in edible meat products reaching the consumer. The work analyzed data from 1,200 cheek meat and head trim tissues from pork carcasses collected bimonthly over a 12-month period from a pork processing plant. The analysis showed high carriage rates of diverse *S. enterica* serotypes from the samples. The majority of isolations occurred during colder months compared with warmer months, and approximately 60 percent of isolates were resistant to three or more antimicrobial drugs. These data point out the potential risk of human exposure to *S. enterica* if these byproduct tissues are incorporated into edible meats, and suggest that further preharvest and postharvest intervention methods should be explored.

Fusarium head blight pathogens and their toxin potential. Fusarium head blight (FHB) is a major disease of wheat, barley, and other cereals that reduces yield and contaminates grain with mycotoxins that can be a significant problem for public health and animal production. A number of different fungi can cause FHB and contaminate grain with a variety of toxins. However, understanding of FHB pathogen diversity, distributions, and toxin potential is incomplete and traditional methods of pathogen identification and toxin analyses are either ineffective or expensive and time consuming. In research with international collaborators, ARS researchers in Peoria, Illinois, identified and described a novel species (Fusarium praegraminearum) capable of causing FHB in wheat and developed new genetic tools to identify strains of this FHB pathogen. A similar set of genetic tools were used to document for the first time that the FHB pathogen F. meridionale is present in wheat fields in North America, and to determine the extent and distribution of different toxin types within a widespread FHB pathogen, F. culmorum. The ability to detect, accurately identify, and determine the distribution and toxin potential of FHB pathogens will allow development and deployment of FHB and mycotoxin control strategies effective against the FHB pathogens and toxin types prevalent in a given region.

Barley sugar transfer gene helps to control Fusarium head blight (FHB). FHB affects small grain cereal crops by causing yield reductions and contamination of grain with nivalenol (NIV) or deoxynivalenol (DON), two known trichothecene mycotoxins. These toxins are harmful to the health of humans and livestock because of their ability to block protein synthesis. Plants infected with mycotoxin-producing fungi can detoxify DON by attaching the toxin to a sugar. ARS researchers in Peoria, Illinois, in collaboration with researchers at the University of Minnesota, identified a gene from barley that produces a sugar-transfer enzyme that efficiently detoxifies DON and NIV. The gene was introduced into an FHB-susceptible wheat variety, and the resulting wheat that expressed the barley gene was significantly more resistant to both the toxins and to FHB. This research represents a critical step toward development of wheat with greater resistance to DON and NIV and serves as a guide for transgenic and traditional breeding approach to increase resistance to DON in cereal crops and thereby improve food safety and crop production.

Elevated carbon dioxide reduces the emission of defense related volatiles from corn. Insect damage allows mycotoxigenic fungi to establish and grow on corn, leading to reduced yields and corn that may be unsafe for humans and other animals. Plants release airborne chemicals (volatiles) in response to infection or attack by the caterpillars of insect pests. These chemicals are used in direct and indirect plant defense. To determine if these key defense signals are altered by changes in climatic conditions, ARS researchers in Peoria, Illinois, and Gainesville, Florida, compared the amounts of caterpillar induced volatiles produced by sweet corn grown at current and elevated carbon dioxide. Corn grown at high carbon dioxide levels released significantly lower concentrations of volatiles than corn grown at ambient carbon dioxide levels. These results suggest corn may become more susceptible to insect pests as atmospheric carbon dioxide levels increase, and identified a trait that can be used in plant breeding programs to improve the natural defense response of corn, which would result in increased yields, reduced mycotoxin contamination of grain, and limited use of pesticides.

Vaccination with live-attenuated vaccine significantly reduces *Salmonella* levels in turkeys. ARS researchers in Ames, Iowa, created a *Salmonella* vaccine with genetic mutations in the bacterial genome that limits *Salmonella* serotype-specific immunity (more than 2,500 *Salmonella* serotypes exist) and induces an immune response that would be cross-protective against diverse *Salmonella* serotypes. The researchers have previously shown the effective reduction of *Salmonella* disease, colonization, and fecal shedding in vaccinated swine. To highlight utility of the vaccine, researchers also tested the vaccine in turkeys that showed a reduction in systemic and intestinal colonization of vaccinated turkeys following challenge with multi-drug resistant *Salmonella* Heidelberg. Pre-harvest control of *Salmonella* in food-producing animals can protect animal health, limit antibiotic usage, decrease environmental contamination, reduce *Salmonella* carriage into the human food chain, and diminish the cost of meat product recalls to producers. The results highlight the utility of the ARS-designed vaccine for enhancing pre-harvest control of *Salmonella*.

Neutralizing Buffered Peptone Water (nBPW) to inactivate chemical sanitizers in Salmonella. ARS researchers in Athens, Georgia, collaborated with the Food Safety Inspection Service (FSIS) to develop a neutralization solution (nBPW) for use by commercial poultry processors in Salmonella verification testing. Commercial poultry processors use chemical sanitizers during processing to reduce contamination of carcasses by human foodborne pathogens. However, if traces of these sanitizers are carried-over into testing samples used to determine contamination of poultry carcasses and parts by Salmonella, the results of these tests may be inaccurate. The utilization of the nBPW improves the accuracy of Salmonella verification testing by inactivating trace amounts of the sanitizers in the test samples. On July 1, 2016, the FSIS informed inspectors in commercial poultry processing facilities that nBPW should be used in all verification testing, and the FSIS is currently seeking a commercial vendor to produce nBPW. The utilization of nBPW will improve the accuracy of verification testing and will provide regulatory agencies and food safety researchers with reliable data to develop methods to reduce contamination of processed poultry by foodborne pathogens.

Colistin resistance gene (*mcr*-1) in U.S. meat animals. Colistin is an antibiotic classified by the World Health Organization as being of critical importance to human health. In 2015, a gene (*mcr*-1) for colistin resistance was found that increases the ease and rate at which resistance can spread to different bacteria. In 2016, ARS researchers in Athens, Georgia, found this bacterial gene in the stomach contents of livestock. From more than 2,000 cecal samples, two isolates of *Escherichia coli* were found that carried the *mcr*-1 gene. Isolates were characterized by whole genome sequencing, resistance profiling, and plasmid mobility studies. The total genomic DNA of the isolates were sequenced, and the U.S. isolates were determined to have descended from isolates found in China, but they were substantially different from each other, so a direct link between them could not be made. This was the first detection of the *mcr-1* gene in the United States, and at this time, it is unknown how the resistance gene traveled from China to the United States.

Fecal shedding of *Salmonella enteritidis* by experimentally infected laying hens. ARS researchers in Athens, Georgia, studied the frequency at which laying hens shed *S. enteritidis* in their feces after being experimentally infected. They found that hens housed in conventional cages shed *S. enteritidis* at a higher frequency than hens housed in enriched colony cages. In recent years, alternatives to conventional cage-based housing for poultry flocks has become more common in the commercial egg industry, but the effects of different housing systems on important public health problems, such as *S. enteritidis* contamination of eggs laid by infected hens, are not yet fully understood. In this study, laying hens were housed in colony cages enriched with perches and enclosed nesting areas at two different stocking densities (defined by the amount of floor space available to each bird), or in conventional cages at a higher density. Both flocks were orally infected with *S. enteritidis*. Samples of voided feces were collected from trays beneath the cages at weekly intervals and tested for the pathogen. The frequency of *S. enteritidis* isolation from feces was significantly greater when infected hens were housed in enriched colony cages at the higher stocking density compared with the lower density, but *S. enteritidis* was isolated from fecal samples at a greater overall frequency from hens in conventional cages than from either group housed in enriched cages. These results demonstrate that hen stocking density can affect the susceptibility of hens to intestinal colonization by *S. enteritidis*.

Development of aflatoxin and fumonisin-tolerant corn lines. ARS researchers in New Orleans, Louisiana, in collaboration with the International Institute of Tropical Agriculture, Nigeria developed six corn varieties (TZAR101-106) with resistance to contamination by the aflatoxin (compounds that are toxic and carcinogenic to humans and animals) producing fungus, Aspergillus flavus. In a recent field trial these six lines also demonstrated resistance to another fungus, Fusarium, responsible for producing a toxin called fumonisin. Using these lines several drought tolerant lines were also developed that will be used by growers in African countries to reduce the incidence of aflatoxin and fumonisin contamination in corn.

Zoonotic bacteria in bulk tank milk. The dairy farm environment is a well-documented reservoir for zoonotic pathogens such as *Salmonella enterica*, Shiga-toxigenic *Escherichia coli*, *Listeria monocytogenes*, and *Campylobacter*, and humans may be exposed to these pathogens through consumption of unpasteurized milk and dairy products. As part of the National Animal Health Monitoring System (NAHMS) Dairy 2014 survey, ARS researchers from Beltsville, Maryland, analyzed bulk tank milk and milk filters from a total of 234 dairy operations from 17 major dairy states for the presence of these pathogens. Prevalence at the farm level was determined (*S. enterica*, 18 percent; *L. monocytogenes*, 3 percent; and *Campylobacter* spp., 25 percent), and genes suggesting the potential presence of Shiga-toxigenic *E. coli* were detected in the milk from 30.5 percent of operations. Resistance to antibiotics may complicate treatment options if humans are infected with bacterial pathogens. Most of the *Salmonella* isolates were susceptible to each of 14 antibiotics used in both human and animal medicine, but 13 percent of the isolates were resistant to 4 to 9 antibiotics. Resistance to tetracycline was detected in 68.4 percent of *C. jejuni* isolates, and resistance to ciprofloxacin and nalidixic acid was detected in 13.2 percent of *C. jejuni* isolates. The results of this study indicate a low but appreciable contamination of raw milk with bacterial pathogens that are known to infect humans, and this information can be used to discourage consumption of raw milk and milk products.

<u>FDA and industry use ARS research to develop food safety practices.</u> Fresh produce processors traditionally have used a specific free-chlorine level [1 ppm (part per million)] as the control limit and a re-wash as the corrective action solutions in hazard analysis and critical control points (HACCP) programs. ARS researchers in Beltsville, Maryland, determined that the industry standard control limit chlorine concentration does not prevent pathogen cross-contamination, and that re-washing of contaminated product is an ineffective corrective action. The research clearly documented significant risk factors associated with generally considered safe operating practices. Follow up studies further demonstrated that a minimum of 10 ppm free-chlorine was required to effectively prevent pathogen cross-contamination during washing. Recommendations have been adopted by leading processors, and incorporated in the interagency and industry taskforce whitepaper entitled “Guidelines to Validate Control of Cross-Contamination during Washing of Fresh-Cut Leafy Vegetables”.

<u>Sensing techniques for food ingredient authentication and food contaminant detection.</u> ARS researchers in Beltsville, Maryland, have developed a new use for Raman spectroscopy called point-scan and line-scan (i.e., Raman chemical imaging) to nondestructively detect chemical contaminants in powdered food materials. Without any special sample preparation, the newest system can analyze a 30-ml sample (2 tablespoons) of dry powder within 10 minutes, more quickly and efficiently than conventional Raman instruments that might require 24 hours to analyze the same sample. The system can perform quantitative contaminant detection down to concentrations as low as 50 parts per million of benzoyl peroxide (used for bleaching) in wheat flour or melamine in milk powder, for example. This system, which is currently under patent review, will provide a useful screening mechanism to help detect contaminated food products and prevent their distribution and use.

<u>Fluorescence imaging for detecting fecal contamination of soil and assessing compost maturity.</u> Serious outbreaks of foodborne illness can result from consumption of fresh produce contaminated by pathogens such as *Escherichia coli* and *Salmonella* that originate from animal or human fecal matter, particularly if the produce is mishandled at temperatures that

encourage pathogen growth. Sources/pathways of contamination can include the excrement of wildlife or livestock, immature manure composts used as soil amendments, contaminated irrigation water or field tools, and poor health or hygiene of field workers. Although food safety standards exist for the use of mature manure composts and prevention of fecal contamination in produce fields, verification in field production settings remains challenging because neither compost maturity nor fecal traces are easily identified by eye. ARS researchers in Beltsville, Maryland, investigated the use of hyperspectral fluorescence imaging techniques to determine the spectral characteristics of fecal samples from four species (dairy cows, pigs, chickens, and sheep) to test animal feces and identify species origin in soil-feces mixtures, and to evaluate the imaging process for use in assessing the maturity of manure-based composts. Results from the study indicate that fluorescence imaging can be used to detect feces on soil and that it is feasible to identify the species of animal from which it originated. Furthermore, the new fluorescence imaging technique is easier to use and produces better results than more complex and costly full-spectrum hyperspectral imaging methods for assessing compost maturity. These findings can be incorporated into field-use practices to help prevent contamination or harvesting of contaminated produce.

Cellphone based technology for bioluminescence detection. Bacteriophage are viruses that infect specific bacteria, and they have been used to detect foodborne pathogens. One such approach has added a light-producing bioluminescent indicator into the bacteriophage that becomes detectable once the bacteriophage has infected its target pathogen. A challenge for this bacteriophage bioluminescence detection is that it requires sensitive and costly instruments to detect the extremely dim light produced. ARS-funded researchers at the Center for Food Safety Engineering (CFSE) at Purdue University, West Lafayette, Indiana, have developed a smartphone-based bioluminescence detection system called BAQS (Bioluminiscent-based Analyte Quantification by Smartphone). The proof of concept was demonstrated with an indicator organism and resulted in the detection of the organism at levels comparable to some currently employed on-site detection methods.

Comparison of Salmonella prevalence between organic and conventional chicken. Salmonella is an important pathogen to control in poultry products, and organic poultry products are becoming more common. Therefore, it is important to determine the effectiveness of current processing methods on Salmonella prevalence in these products. ARS-funded researchers at the CFSE, Purdue University, West Lafayette, Indiana, collected samples from organic and conventional chicken during processing at a commercial facility and determined the prevalence of Salmonella at various processing steps. Results showed that Salmonella prevalence was higher in organic chickens compared to conventional chickens during early processing steps, but prevalence was the same for both processing methods after the final chill step. This indicates that organic raised chickens are similar to those conventionally raised.

High-resolution, low-powered, networked, time-temperature monitoring sensor. Food products stored at improper temperatures are one of the greatest causes of foodborne disease outbreaks. Regulatory efforts are focused on food production, but there is a lack of control measures outside the production plant. Therefore, distribution and retail are considered the weakest links in the food safety management system when it comes to temperature abuses, creating a pressing need for new management mechanisms. Correlation of microbial growth profiles to temperature history requires a sensor that can collect and transmit measurements without interfering with the conditions of the sample under study. To achieve this, ARS-funded researchers at the Center for Food Safety Engineering at Purdue University in West Lafayette, Indiana, developed time-temperature monitoring (TTM) sensors that take temperature measurements with high resolution and transfer them wirelessly to a base station. The sensors have been calibrated to a wide range of temperatures (−40 to 140°F) and have exhibited resolution better than 2°F. These TTM sensors are ready to be used and have the potential to assist in efforts to monitor and manage the environmental conditions to which food products are exposed during distribution and retail, thereby providing crucial information on the quality and handling of food products.

<u>Packaging films releasing antimicrobial vapor.</u> Controlled release of volatile antimicrobials exhibits more antimicrobial efficacy than their liquid phase against foodborne pathogens or spoilage bacteria in fresh produce. ARS researchers at Wyndmoor, Pennsylvania, teamed with a collaborator, developed antimicrobial polylactic acid (PLA) films releasing allyl isothiocyanate (major component in mustard oil) vapor. The antimicrobial film had more flexibility, lower gas permeability, and higher UV blocking ability than pure PLA film. The vapor released inhibited bacterial growth in fresh vegetables stored at 4 and 10 degrees C for 15 days and has the potential to be utilized for extending the shelf-life of various perishable fresh produce.

<u>Analytical method for veterinary drug residues in liquid and powdered eggs.</u> The Food Safety and Inspection Service (FSIS) is responsible for monitoring the safety of liquid and powdered egg products, including veterinary drug residues, to assure that regulatory tolerances are not exceeded. However, FSIS lacked a validated analytical method that could be used to determine the presence of veterinary drug residues. To address this lack of information, ARS researchers in Wyndmoor, Pennsylvania, extended and validated their analytical method for food animal tissues to egg products. The results met FSIS validation criteria for more than 150 drug residues in the samples, and the method was transferred to FSIS for routine use in their monitoring laboratories.

<u>New sorbent material for efficient cleanup of complex food samples.</u> Chemical analysis of residual contaminants in highly complex pigmented and fatty foods requires efficient cleanup of the food extracts to remove interfering matrix components. ARS researchers at Wyndmoor, Pennsylvania, evaluated a new commercial sorbent, called Verde, which combines fat and pigment removing properties, in fatty and/or pigmented commodities (avocado, pork, salmon, and pork) for analysis of 117 pesticides and environmental contaminants. The new sorbent provided efficient removal of chlorophyll and lipids, which enabled acceptable analysis of nearly all of the selected contaminants at or below regulatory tolerance levels. The new method is fast, efficient, and can be implemented for residual analysis of contaminants in foods.

<u>The USDA Integrated Pathogen Modeling Program (IPMP)–Global Fit.</u> Mathematical models are frequently used to predict the growth and survival of microorganisms in food throughout the

supply chain, and are the foundation of quantitative microbiological risk assessment. Accurate estimation of kinetic parameters is essential to predictive modeling. ARS researchers in Wyndmoor, Pennsylvania, have expanded the USDA IPMP–Global Fit, a one-step kinetic analysis software for predictive modeling. Growth and survival models are included in the new software for direct construction of predictive models that minimize the global errors. This new approach to mathematical modeling can significantly improve the accuracy of data analysis and future model development. IPMP–Global Fit is free for researchers and risk modelers around the world to use and can be downloaded from this web address: www.ars.usda.gov/northeast-area/wyndmoor-pa/eastern-regional-research-center/docs/ipmp-global-fit/.

Food safety at grocery stores. Retail grocery store shoppers and employees view food safety risks differently than food safety experts do, and as a result, they may be at higher risk for becoming sick. In collaboration with researchers at North Carolina State University, ARS researchers in Wyndmoor, Pennsylvania, collected about 120 digital photographs at grocery stores in California, Connecticut, Georgia, and Maryland of both possible and actual food safety risk situations. As examples, photographs captured utensils such as tongs placed handle-down in containers of uncovered ready-to-eat foods, bare-handed contact of delicatessen meat during slicing, and water dripping from a ceiling onto a food-preparation counter. These digital photographs can be used as a motivation and as a real-world teaching lesson to better inform shoppers and employees at grocery stores of good practices.

Cold plasma improves food safety. Cold plasma is a non-thermal processing technology that uses reactive gases to inactivate foodborne pathogens. ARS researchers in Wyndmoor, Pennsylvania, conducted various studies showing the following results: 1) Cold plasma treatment for 3 minutes eliminated spoilage yeasts and molds, and reduced *Salmonella* levels by up to 99.95 percent on grape tomatoes packaged in a polyethylene terephthalate commercial clamshell container. This improvement in safety and storability was achieved with no adverse effect on tomato color, firmness, water content, pH, total soluble solids, or lycopene concentration; 2) In apples, a chemical sanitizer combined with cold plasma reduced *Listeria monocytogenes* levels by greater than 99.99 percent, even in the calyx area; 3) Cold plasma applied to blueberries inactivated two widely used surrogates for human norovirus, Tulane virus and murine norovirus. A treatment of 45 seconds reduced Tulane virus levels by 96 percent, with 99.97 percent reduction after 120 seconds. Murine norovirus was eliminated (>99.999 percent) after only 90 seconds; and 4) Cold plasma treatment inhibited levels of *Escherichia coli* O157:H7, *Salmonella* spp., *L. monocytogenes*, and Tulane virus by 92 percent, 60 percent, 90 percent, and 95 percent, respectively, on Romaine lettuce irrespective of packaging atmosphere humidity content. Both rigid and flexible conventional plastic packages appear to be suitable for the cold plasma decontamination. The results show that with further optimization, cold plasma may be used by food processors as a new method to improve the safety of fresh and fresh-cut fruits and vegetables, including fragile produce such as berries.

Inactivation of uropathogenic Escherichia coli (UPEC). High pressure processing, gamma radiation, and ultraviolet light are sustainable food safety technologies that can kill harmful bacteria in meat and poultry. Uropathogenic Escherichia coli are a common contaminant in poultry meat and are now emerging as being associated with urinary tract infections. These infections affect over 10 million people each year, primarily women resulting in over 23,000 deaths. ARS researchers at Wyndmoor, Pennsylvania, found that high pressure processing (500 MPa, 4°C, 4.43 min), gamma radiation (1.3 kGy at 4°C or 1.6 kGy at -20°C), or ultraviolet light (125 mJ/cm2) killed 99.999% of UPEC in chicken meat or chicken purge. The results of this study will allow regulatory agencies and food processing industries to conduct risk analysis and provide safer poultry meat to consumers. Consumers, especially those who are immuno-compromised will benefit from having more information about foods treated with alternative processes which kill harmful bacteria.

New Vibrio predatory bacteria. Vibrio bacteria, particularly Vibrio vulnificus and Vibrio parahaemolyticus, cause illnesses and deaths to consumers of raw or partially cooked shellfish. Processing interventions are needed to kill vibrios in shellfish to enhance seafood safety. ARS Researchers at Dover, Delaware, identified three novel marine bacterial strains (Pseudoalteromonas piscicida) as predators of human pathogenic vibrios. These bacteria effectively reduce vibrios and other human pathogens in the marine environment and may provide the key to the development of processing interventions to reduce shellfish-associated illness.

Method validation to enhance human norovirus detection. Most shellfish contamination by human noroviruses is from faulty sewage treatment processes and the release of large quantities of norovirus into the coastal environment. A method developed by ARS Researchers at Dover, Delaware, known as the PGM-MB binding assay, detects viable human noroviruses and has been applied to evaluate the efficacy of sewage treatment. Results demonstrate that human norovirus is not inactivated by chlorine-based sewage treatment. This data has been provided to FDA as part of their ongoing shellfish risk assessment project and may affect the way the FDA regulates the harvesting of shellfish.