FY 2015 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

Problems Statements

1. A Population Systems

This area identifies and characterizes the movement, structure, and dynamics of populations throughout food production, processing and storage; hence the entire safety continuum. Major components of emphasis and interaction include epidemiology, ecology, host-pathogen relationships.

Anticipated products

- Epidemiologic studies will provide a scientific approach for population-based studies on new detection methods and interventions, to design and evaluate risk factors for potential control or intervention strategies, and a framework to integrate genomic data with disease in populations.
- Ecologic studies will determine the attributes and changes in the ecological communities in order to understand the transmission and dissemination of pathogens and toxins in and among food producing animals and crops, and the interactions and relationships within the population community.
- Host-pathogen relationship studies will 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; the role of protozoa in harboring or transmitting bacterial foodborne pathogens (Trojan horse concept); and the role of commensals.

1. B. Systems Biology

The concept of systems biology involves a unique integrative approach to understand the basic genetic components of pathogens, their expression, and directly relate this information to the microorganism's biology.

Anticipated products

- This approach provides a unique opportunity to understand the basic genetic components of pathogens, their expression, and directly relate this information to the microorganism's biology.
- While the tools for gene expression studies are available, there needs to be an increased focus on understanding how the studies will be performed and interpreted, and how they can be used to promote food safety.
- Establish a metagenomics approach to selected research areas which will for example, allow determination of metabolic contributions to risk.

1. C. Technologies for the Detection and Characterization of Contaminants

Challenges arise from either uncontrolled microbes entering through raw materials, contamination during processing, or from undesired chemical contaminants including chemical residues, and bacterial, fungal and plant toxins. [Sensitive and specific] detection technologies are required at the earliest possible stage in the food chain, thus avoiding/preventing the need for processing interventions, or possible recall.

Anticipated products

- Promising technologies will be advanced. Technology transfer has to be done quickly, and where possible, and appropriate, will undergo validation through national or international bodies (FERN, Codex).
- Research that offers minimal outcome or impact will be terminated, and alternate approaches formulated. For example, detection methods related to serotyping and subtyping pathogens are useful; however, stronger emphasis must be placed on methods for more effective identification.

- Development of technologies must yield method(s) that are faster and yield improved resolution.
- In developing technologies decisions cannot be made in isolation. There needs to be an integration of biology, epidemiology and the physical sciences systems.

1. D. Intervention and Control Strategies

To ensure safe food and protect public health, intervention and control strategies must be identified, implemented, and then measured as to their impact on the reduction and control of food-borne pathogens or other zoonotic organisms, and chemical contaminants. This approach incorporates strategies in both pre- and post-harvest systems as the science dictates, to produce a complementary and efficient approach for food safety.

Anticipated products

- Intervention strategies will be developed to eliminate and/or control microorganisms in animals and their derived products, seafood and plant production, processing and storage systems. An underlying assumption is that production control interventions reduce downstream contamination which subsequently reduces disease risk.
- Efforts will focus on developing environmentally compatible technologies.
- Strategies will be developed for operations of all sizes (large to very small).
- Pathogens may develop resistance to some interventions; thus, efforts should focus on development of combinations of new or innovative intervention technologies for (minimal) processing.
- Interventions will be developed 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

1. E Predictive Microbiology

The behavior of any microorganism is deterministic and able to be predicted from knowledge of the microorganism itself, and the microorganism's immediate environment. Behavioral predictions are an integral part of microbial risk assessment used to support food safety measures.

Anticipated products

- The ARS Food Safety 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.
- The Program conduct research and provide data when requested by our regulatory stakeholders (FSIS, FDA) for their use in conducting risk assessments.
- Collaborations with regulatory and public health agencies will be strengthened regarding research for RA development efforts, so as to effectively utilize the inherent ARS expertise and modeling mechanisms.
- Methods used to identify data gaps will be described and integrated into the research project.
- Data acquisition will be an ambitious interdisciplinary research challenge that will eventually translate into improved public health.

1. F. Chemical and Biological Contaminants: Methodology, Toxicology and Toxinology

The regulation and control of veterinary drugs, 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 human health and the environment.

Anticipated products

- The successful implementation of technologies developed and validated through research is the major goal.
- These technologies provide tangible benefits through a more effective and efficient means of monitoring the food supply, and environment where food is grown. Better methods assist researchers conducting toxico/ toxinological studies.
- Toxico/toxinological studies provide basic and applied knowledge on the effect of exposure to biological toxins.

Selected Accomplishment for FY 2015

Residue detection. Ensuring that surfaces are effectively cleaned and sanitized is critically important for the food industry. Because cross-contamination from surfaces by pathogenic bacteria can lead to foodborne illnesses, handheld imaging devices are needed as aids to detect food residues on processing surfaces. A handheld fluorescence imaging device developed by ARS scientists in Beltsville, Maryland, was validated for its ability to detect food residues on spinach leaf, milk, and bovine red meat, three foods that have been associated with foodborne illness outbreaks. Two common processing surfaces (high-density polyethylene and food-grade stainless steel) were evaluated. Interchangeable optical filters were selected to optimize the contrast between food residues and processing surfaces as they were detected using hyperspectral fluorescence imaging. Fluorescence imaging and analysis helped to more clearly differentiate food residues from the processing surfaces than did human visual inspection in ambient lighting. This cost-effective optical sensing device can be used over relatively large or complex surfaces of processing equipment to detect food residues and has potential for use in the food industry as an aid for detecting specific (targeted) food residues.

Environmental Protection Agency (EPA) registration of new ARS aflatoxin biocontrol product. The use of biopesticide strains that do not produce a toxin (atoxigenic) to prevent aflatoxin contamination has expanded in the United States to include cotton, corn, peanut, fig, and pistachio acreage. This expansion has resulted in the need for additional capacity to produce these biological control agents. To meet the demand, ARS researchers in Tucson, Arizona, developed novel biocontrol formulations that permit high production while reducing capital input, lowering energy costs, and using less expensive materials. With the Arizona Cotton Research and Protection Council (ACRPC) and the IR-4 project, ARS submitted multiyear field efficacy, safety, stability, quality, and manufacturing performance data to EPA as part of a request for registration for a biopesticide identified by ACRPC and named *Aspergillus flavus* AF36 Prevail. Based on that data, EPA granted an unconditional registration for the new biopesticide in June 2015. This unconditional approval will allow cost-effective increases in the use of atoxigenic strain-based biopesticides to reduce aflatoxin contamination in the United States.

Antimicrobial resistant *Escherichia coli* and *Salmonella* in cattle. Concerns have been raised that antimicrobial resistant (AMR) *E. coli* and AMR *Salmonella* species may be present in cattle feedlots, persist through beef processing, and contaminate final products. In addition, it has been theorized that meat products, including beef products, are a source of extraintestinal pathogenic *E. coli* (ExPEC) that cause human urinary tract infections. ARS scientists in Clay Center, Nebraska, tracked the occurrences of AMR *E. coli*, AMR *Salmonella*, and ExPEC in cattle from feedlots through processing. AMR *E. coli* were present on 100 percent of hides at feedlots, on 100 percent of hides when cattle began processing, on 1 percent of final carcasses, and on 0 percent of final products. AMR *Salmonella* species were identified on 11 percent of hides at feedlots and on 8 percent of hides when cattle began processing but not on carcasses or final products. ExPEC were rarely detected (0.4 percent) in feedlot and preintervention processing, and ExPEC were never detected from postintervention processing and final products. These results, conveyed to industry and regulatory agencies, indicate that sanitizing interventions currently employed at beef processing plants effectively eliminates AMR bacteria and ExPEC from final products.

Vaccine to control pathogenic bacteria in poultry. The poultry and animal industries continue to combat the spread of foodborne pathogens in food products and have spent millions of dollars attempting to control *Salmonella* and *Campylobacter* with minimal results. Poultry companies have ruled out many intervention strategies because of costs. New interventions must be cost-effective and easily integrated into normal production practices. ARS researchers in College Station, Texas, demonstrated that a normal Marek's disease vaccine can increase the *Salmonella* load in birds if given incorrectly. However, by vaccinating the chick embryo on day 18 of incubation instead of the day of hatch, *Salmonella* can be reduced 10-fold in the chick. This is a significant effect; the results have attracted interest from several U.S. vaccine companies. Additional studies have been conducted with the infectious bronchitis vaccine.

"Democratized" *Salmonella* serotyping. A democratized assay is a bacteriological assay that could have a major effect on improving public health and thus should be developed to have low cost and wide availability. Determining *Salmonella* serotypes can be an expensive and time-consuming effort; however, serotyping is a fundamental method that identifies those strains with most risk to the food supply. More than 2,600 serotypes of *Salmonella* have been identified but only 30 are considered to be recurring causes of foodborne illness. Although DNA-based serotyping has been used by research laboratories for some time, no one technique has made it to the point at which a producer could have access at an affordable cost until ARS researchers in Athens, Georgia, were able to democratize *Salmonella* serotyping. ARS obtained approval to release a DNA database for assigning a serotype to *S. enterica* in the USDA Open Data Catalog. To facilitate open use by industry, regulatory agencies, and food safety monitoring services, ARS also provided a set of 96 key isolates to Neogen Corporation for development of a streamlined service for serotyping (www.neogen.com/Corporate/PR2015/2015-07-27.html). Having an innovative and rapid serotyping method will alleviate many of the current typing problems.

Salmonella detection for Food and Drug Administration (FDA). *Salmonella* contamination of foods regulated by the FDA is a critical concern to ARS. Two technologies developed by ARS-funded scientists at the Center for Food Safety Engineering at Purdue University were among the

five finalists in the first-ever FDA Food Safety Challenge 2015. The innovation challenge presented by FDA was to encourage development of technologies for detecting *Salmonella* in minimally processed fresh produce. The grand prize-winning bioseparation technology developed by Purdue is a physical method for concentrating *Salmonella* to detectable levels using a combination of enzyme treatment and prefiltration followed by automated microfiltration. The sample preparation is completed within 4 hours, leaving another 4 hours to accomplish specific pathogen detection within a work shift (8 hours). The Purdue team plan to continue working with FDA scientists to further develop the prototype system into a commercial system for food safety laboratories to use in detecting *Salmonella*.

Clostridium perfringens cooling models. The bacterium *Clostridium perfringens* is a major foodborne pathogen of concern because it produces an enterotoxin with superantigenic activity that causes extreme foodborne illness and subsequent sequelae. A particular issue that concerns FSIS is that *C. perfringens* has an extremely fast growth rate, with a generation time of less than 10 minutes. After meat products have been cooked to achieve desired lethality for vegetative foodborne pathogens, without adequate and rapid cooling of the product, any heat-resistant spores of *C. perfringens* can germinate and grow in a nutrient-rich environment without bacterial competition. FSIS regulations require food establishments to use models that predict *C. perfringens* growth to document process compliance. ARS researchers in Wyndmoor, Pennsylvania, compared several well-known models and found that three models can be considered useful and reliable tools for food processors and regulatory agencies for evaluating the safety of cooked/heat-treated, uncured meat and poultry products involved in cooling deviations or developing customized cooling schedules.

Downy mildew disease promotes the multiplication and persistence of *Escherichia coli* O157:H7 on lettuce. Lettuce is one of the main crops implicated in produce-linked outbreaks of *Escherichia coli* O157:H7 infections. In the Salinas Valley of California, one of most important lettuce growing region in the United States, lettuce is also frequently infected with the downy mildew pathogen (a fungus-like water mold). ARS scientists in Albany and Salinas, California, determined that *E. coli* O157:H7 multiplied 1,000-fold more in downy mildew lesions than on healthy lettuce leaf tissue under warm temperature and on wet leaves. On dry lettuce leaves, the pathogen persisted in greater numbers when downy mildew disease was present. A lettuce line that is more resistant to the downy mildew pathogen supported less *E. coli* O157:H7 multiplication than those of a line that was highly susceptible to the pathogen. This and related findings indicate that breeding lettuce against downy mildew, which is one of the biggest problems that lettuce growers must manage, may also serendipitously be effective as a defensive line against *E. coli* O157:H7 colonization.

Antimicrobials to decontaminate pathogens on fresh beef. Although numerous interventions targeting *Escherichia coli* O157:H7 have been developed and implemented to decontaminate meat and meat products during the harvesting process, the beef industry still seeks novel compounds that effectively reduce or eliminate non-O157 Shiga toxin-producing *E. coli* (non-O157 STEC) and *Salmonella*. ARS scientists in Clay Center, Nebraska, worked with antimicrobial compound suppliers and beef industry partners to evaluate four compounds: hypobromous acid, neutralized acidified sodium chlorite, Citrilow, and FreshFx, all of which are approved by the FDA. The results indicated that these commercially available compounds

effectively reduced up to 99 percent of *E. coli* O157:H7 and *Salmonella*, and were effective against the non-O157 STEC. These compounds can be used alone or integrated into multihurdle systems to enhance safety and wholesomeness of fresh beef.

Lead and arsenic in soils. Lead and arsenic are two heavy metals of significant concern to the food industry (baby food producers, in particular) and public health regulatory agencies. Lead-arsenate was used as a pesticide in apple orchards from the 1900s to 1960s, and residual lead and arsenic may remain in those soils. Some of the lands are now being converted to vegetable crop production, and because of food safety concerns about lead in carrots, the FDA asked ARS to investigate the nature of the lead. ARS scientists in Beltsville, Maryland, grew three cultivars of carrots on four lead-arsenate-contaminated soils to determine lead and arsenic uptake. Studies showed that lead was significantly higher in peeled carrots compared with the peel, whereas arsenic was highest in carrot peel than in peeled carrots. These findings, relayed to the FDA and farmers, provides valuable information on the safety of alternative use of old orchard soils.

Low-cost, portable optical detector for Shiga toxin 2 (Stx2). Shiga-toxigenic *Escherichia coli* (STECs), in particular subtype 2 strains, are a major cause of foodborne illness and responsible for many deaths worldwide. To reduce the sources and incidence of foodborne illness caused by STEC, a need existed to develop field-deployable sensitive devices to detect active Stx2. ARS scientists in Albany, California, collaborated with FDA scientists to develop a low-cost, simple, portable, optical sensor detection system to measure Stx2. The sensor measures a fluorescent signal produced in the presence of toxin. The system was evaluated side-by-side with a commercial laboratory instrument and it performed better. The prototype sensor system could detect active toxin as low as 0.1 pg/ml and will fulfill the need for portable onsite tests for Shiga toxins. More critical is that the sensor system can be purchased with off-the-shelf components for $300 compared with a current fluorometer costing $35,000. The camera sensor system can be easily adapted to detect other foodborne toxins that may have long-term diagnostic role for developing countries.

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A novel method to detect aflatoxin M_1 in milk. Aflatoxin B_1 produced by the fungus *Aspergillus* is a highly carcinogenic mycotoxin that affects humans, and it is also commonly found in feed used for dairy cattle/milk production. Instead of being excreted, B_1 can, unfortunately, be metabolized by dairy cattle into another carcinogen, aflatoxin M_1 (AFM1), which is excreted in their milk. Detecting aflatoxins in milk is a critical issue for the dairy and related food industries and for the FDA, which regulates milk production. To test milk samples, ARS scientists in Peoria, Illinois, developed a novel technique that combines mass spectrometry with an ambient ionization method. The procedure, known as direct analysis in real time-mass spectrometry (DART-MS), allows rapid and sensitive testing of milk samples without extensive sample preparation or time-consuming chromatographic separation. This is a new rapid, sensitive, and convenient analytical tool for industry and the FDA to assure the safety of milk-based food products.

Salmonella elimination from aerated wastewater in dairy lagoons. *Salmonella* is the most commonly identified foodborne pathogen in produce, meat, and poultry. Cattle are known reservoirs of *Salmonella,* and when the pathogen is excreted in feces, it ends up in manure flush lagoons. ARS researchers in Albany, California, demonstrated that populations of *Salmonella* species declined rapidly with reduction times of less than 2 days in aerated wastewater prepared from lagoons equipped with circulating aerators when compared with nonaerated waters, and with greater reductions in summer than winter. The results indicate that holding wastewater for sufficient time in lagoons with aerators yields pathogen-free, nutrient-rich water, which is important because some growers use dairy lagoon water to irrigate fodder plants and crops and as water for dairy cows, and as fertilizer.

Chlorine dioxide residues defined. Chlorine dioxide (ClO_2) gas can be used as a chemical intervention to significantly reduce pathogenic and rot organisms on produce. However, its regulatory approval by Federal agencies has been precluded by the absence of conclusive residue data. ARS researchers in Fargo, North Dakota, used labeled ClO_2 gas to provide definitive studies of ClO_2 residue fate and distribution on tomatoes and melons. The studies showed that the major residue remaining on plant tissues is chloride, a nutrient present in all foods. Researchers also provided data demonstrating how the formation of unwanted residues (perchlorate, for example) may be prevented. The data have been submitted to the EPA for review. If approved, ClO_2 sanitation of vegetables, melons, and other fresh produce could be implemented and play a significant role in ensuring that fresh produce is free of pathogens. Reducing pathogens on produce is a critical issue because produce accounts for nearly 50 percent of foodborne illness outbreaks.

Transmission of *Salmonella*. Vertical transmission (mother to offspring) of *Salmonella* contributes to persistent, carrier-type infections in cattle. *Salmonella* is an important foodborne and animal pathogen that can reside within the gastrointestinal tract and more importantly, within the lymph tissue of food-producing animals. ARS researchers in College Station, Texas, working in cooperation with researchers at Texas Tech University, demonstrated vertical transmission of *Salmonella* from pregnant cows to their neonatal offspring. Results demonstrated that Holstein dairy calves can be born with *Salmonella* in multiple tissues (gastrointestinal, lymph nodes, organs). These findings challenge the traditional view that *Salmonella* infection occurs exclusively via the fecal-oral route of transmission. They also

provide an explanation for depressed immune-recognition of *Salmonella* in persistently infected cattle. This research provides critical new information on transmission dynamics of *Salmonella* that can lead to the development of new pathogen intervention strategies to help producers continue to produce *Salmonella*-free meats.

Inflammatory response alters drug residue composition. Chemical residues in foods for human consumption are a critical food safety issues for FSIS. Unfortunately, dairy cattle contribute significantly to the total burden of drug residue violations in the United States. ARS scientists in Fargo, North Dakota, undertook a study to determine whether drug residues were related to the route of drug administration or the presence of inflammation (to mimic infection). The study showed that composition of metabolites in liver and urine were correlated with residue concentrations in liver, but only in cows undergoing an inflammatory response. This work provides a rationale for why dairy cattle have a relatively high incidence of unsafe levels of residues suggests to the dairy industry that drug clearance could be altered in dairy cattle being treated for inflammatory conditions through better, more effective production management.

Reducing fumonisin exposure in developing countries. Fumonisin-producing molds are commonly found in corn, and consumption of contaminated corn by farm animals has been shown to be the cause of disease. Fumonisin intoxication has also been hypothesized to be an environmental risk factor for diseases in humans in countries where corn is a dietary staple and infection with the mold is likely. To determine whether fumonisin contributes to disease in humans, ARS scientists in Athens, Georgia, in collaboration with researchers at Centro de Investigaciones en Nutrición y Salud in Guatemala, Creighton University, and Duke University developed methods to measure changes in the urine and blood levels of chemicals that are indicators of changes indicative of predisease states. Human studies conducted in Guatemala where corn is a dietary staple showed that fumonisin intake and changes in a unique class of fats (sphingoid base 1-phosphates) in the blood are correlated in a manner that mimics the effects of fumonisin in laboratory animal findings. The findings are consistent with the hypothesis that fumonisin inhibits the same enzyme in humans as it does in farm and laboratory animals that consume diets high in fumonisin. These studies are critically important for medicine because they are the basis for development of biomarker-based studies designed to identify possible human diseases where fumonisin could be a contributing factor. Furthermore, the data will provide an incentive to reduce fumonisin exposure in developing countries where corn is a dietary staple.

Preventing spoilage of fermented vegetables. Research studies did not show the significance of *Bacillus* spoilage of acidified vegetables is a significant problem for industry, especially tomato processors. In 2010, the FDA had proposed guidance for the food industry stipulating that spores of *Bacillus* species needed to be killed by heat processing for the production of acidified vegetable products. Implementing the guidance would have been a significant burden to industry because the times and temperatures needed to thermally process these products would negatively affect product texture and quality. ARS scientists in Raleigh, North Carolina, undertook research to show how the different acids present in tomato vs. acidified vegetables (such as cucumber pickles, and peppers) affect the spoilage process. The acids added to preserve acidified vegetables (primarily vinegar) are sufficient to prevent *Bacillus* spoilage, whereas the malic acid naturally present in tomato products is not necessarily sufficient to prevent spoilage. These data

were made available to the FDA and industry representatives for the development and implementation of science-based regulations and production practices.

Novel device for pathogen inactivation. Monitoring the effectiveness of a sanitizer during food processing is a critical component of a Hazard Analysis Critical Control Point plan. ARS scientists in Beltsville, Maryland, developed, fabricated, and validated a novel microfluidic mixer to accurately determine time-dose responses of pathogen inactivation with chlorine solutions from 0.1 second to 5 minutes. This invention provides a valuable tool for industry and public health agencies to determine pathogen inactivation kinetics in the subsecond time scale, filling a void in laboratory equipment that had been needed to study pathogen inactivation kinetics in fractions of a second. The technology was transferred to CDC and is currently in use.

New edible food coating. The contamination of ready-to-eat meat products by foodborne pathogens is a concern for the meat industry. One of the potential solutions to prevent these pathogens is to wrap meats in films composed of natural biopolymers combined with nanotechnology. ARS researchers in Wyndmoor, Pennsylvania, developed edible antimicrobial composite films from microemulsions containing all-natural compounds using high-pressure homogenization (HPH) technology. HPH treatment significantly reduced polymer particle sizes in the emulsion to about 1 mm. The films from the coating solution were softer, less rigid, and more stretchable than those without HPH treatments. When the composite films were used in experimental studies that mimicked industry food processing conditions, *Listeria* on the surface of ready-to-eat meat samples was inactivated by 99.99 percent after 35 days at 10°C. These new edible antimicrobial films and coatings could be used by industry as an intervention to enhance the safety of ready-to-eat foods.

Prevalence and risk factors of *Toxoplasma gondii* infection. *T. gondii* is a protozoan parasite that is responsible for approximately 24 percent of all estimated deaths attributed to foodborne pathogens in the United States. Human infection results from accidental ingestion of oocysts either through water or through insufficiently washed produce, or via consumption of raw or undercooked meat products that contain *T. gondii* tissue cysts. ARS scientists in Beltsville, Maryland, working with researchers at the CDC reviewed studies of *T. gondii* infection in meat because substantial proportions of human *T. gondii* infection are acquired through consumption of raw or undercooked meat. Prevalence of *T. gondii* is higher in conventionally reared pigs, sheep, and poultry than it is in cattle. Prevalence of *T. gondii* is greater in meat products from organic compared with conventionally reared meat animals because access to the outdoors poses substantially greater opportunities for animal exposure to infected rodents, wildlife, and oocyst-contaminated feed, water, and environmental surfaces. Risk factors related to *T. gondii* exposure for livestock include farm type, feed source, presence of cats, methods of rodent and bird control, carcasses handling, and water quality. This work serves as a useful resource and information repository for informing quantitative risk assessment studies for *T. gondii* infection in humans through meat consumption.

Dairy cows and pathogenic *Listeria*. Some *Listeria* species are known human pathogens that cause foodborne illness and potentially severe sequelae. There is considerable known diversity within serotypes (strains) of *L. monocytogenes*, and their potential to cause disease in humans is also variable. To better understand the relationship between strains found on dairy farms and

those involved in human illness, ARS researchers in Beltsville, Maryland, examined the diversity of *L. monocytogenes* isolates collected over a 6-year study from the feces of dairy cattle on a single dairy farm. Differences were assessed using a multivirulence locus sequence typing assay. The results were compared with those from other strains isolated globally from human clinical cases, foods, and the environment. Results demonstrated that multiple, distantly related *L. monocytogenes* strains persisted among members of the herd over the course of the study, whereas other strains were present for short time periods only. Some strains isolated during this study were identified as new sublineages in the *L. monocytogenes* global phylogeny, whereas others were closely related to epidemic clones that had been previously isolated from human clinical cases. The importance of this work is that it demonstrated to dairy producers, processors, and regulatory agencies that dairy cows can be reservoirs of a diverse population of human pathogenic *L. monocytogenes*, which can be a potential risk to consumers of milk, dairy products, and meat.

National survey of sheep for *Salmonella* prevalence. Very little is known about the occurrence of *Salmonella* in sheep, how often the organism is associated with disease in sheep, and whether antimicrobial resistance genes flow through this population. ARS scientists in Athens, Georgia, collaborated with APHIS to determine how often sheep are infected with *Salmonella*, characterize the salmonellae that were found for their type, and determine whether they were resistant to antimicrobial treatment. *Salmonella* collected from sheep nationwide were evaluated. Approximately 4,000 fecal samples were collected from 247 farms in 22 States. Seventy-two percent of the farms had at least one sample positive for *Salmonella* and 27 percent of all the samples were positive. Ewes that were nursing lambs were most likely to be infected with *Salmonella*. Antimicrobial resistance was rare among the observed isolates. The vast majority (95 percent) of the *Salmonella* that were isolated were of the type known as *S. enterica* subspecies diarizonae, a rare type outside of sheep populations. Results of this survey indicate that *Salmonella* species typically found in sheep are not likely to be associated with foodborne disease in the United States.