**FY 2008 Annual Reports for National Program 108, Food Safety**

**Executive Summary**

Food Safety falls under Goal 4 of the Agency Strategic Plan, to Enhance Protection and Safety of the Nation’s Agriculture and Food Supply. The program’s mission is to provide, through scientific research, the means to ensure that the food supply is safe and secure for consumers and that food and feed meet foreign and domestic regulatory requirements. To ensure a secure agricultural system, ARS works to reduce or eliminate factors that threaten the ability of U.S. agriculture to produce enough food, year-to-year, to meet the needs of American consumers. Research is designed to generate knowledge regarding new and improved management practices, pest management strategies, sustainable production systems, and control of potential contaminants for farms of all sizes. In particular, 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. Since food safety and food security are global issues, our research program involves both national and international collaborations through formal and informal partnerships. Our accomplishments and outcomes are utilized in national and international strategies delivering research results to regulatory agencies, commodity organizations, and consumers to support further efforts to provide a secure, affordable, and safe food supply.

The program’s vision is to increase public health through the development of technologies that protect food from pathogens, toxins, and chemical contamination during production, processing, and preparation, thus increasing the safety of the food supply. The research components of the program during 2008 were:

* [Microbial] Pathogens, Toxins, and [non-biological-based] Chemical Contaminants, subdivided into Pre-harvest and Post-harvest
* Mycotoxins and Plant Toxins

**Program Highlights**

During 2008, there were several significant events in the Food Safety National Program. Dr. Mary Torrence joined the Office of National Programs to jointly oversee the program after her previous position as National Program Leader for Food Safety at the USDA-Cooperative State Research, Education, and Extension Service, where she oversaw the Food Safety Epidemiology Program. Also, draft outlines were developed for the NP 108 2011-2015 Strategic Plan and NP 108 2011-2015 Strategic Action Plan, which will form the basis for food safety research in the next Office of Scientific Quality Review (OSQR) cycle. In 2009, the National Program will undergo an external Retrospective Review and a Stakeholder Workshop prior to preparation of the next OSQR research cycle.

**Selected Research Highlights**

Detection of melamine. The detection of melamine-contaminated imported food products is a critical issue for the Food and Drug Administration (FDA). ARS scientists developed a rapid, nondestructive detection/identification method for melamine and its derivatives in pet foods, based on Raman spectroscopic techniques. A patent disclosure was approved for the method, and a Cooperative Research and Development Agreement (CRADA) was initiated, resulting in the development of two prototype hand-held devices currently undergoing testing and validation in commercial settings. This work will have a direct impact on the FDA’s ability to detect melamine and related contaminants in foods.

Quantitative reduction of *Listeria monocytogenes* using bacteriophage. New classes of GRAS (generally recognized as safe) antimicrobials are in strong demand as mitigation strategies against *L. monocytogenes* in various ready-to-eat food products. The FDA has recently approved two lytic bacteriophage preparations as GRAS for the protection of ready-to-eat food products against the bacteria. In collaboration with scientists at Mississippi State University, ARS scientists have developed models to determine the efficacy of the bacteriophage preparations against *L. monocytogenes* in aquaculture products. This research validates new methods for controlling Listeria in ready-to-eat foods, and will allow the FDA to develop better risk assessments.  
  
Persistence of *Escherichia coli* on spinach leaves evaluated. The contamination of leafy-green, fresh cut produce is a major food safety concern. ARS scientists examined how long *E. coli O157:H7* could survive under field conditions on spinach leaves, comparing the bacteria’s survival on spinach plants and in organic soil in a growth chamber that simulated field conditions. Populations of *E. coli O157:H7* survived for a shorter duration on spinach shoots than in soil, with non-pathogenic *E. coli* detected intermittently on spinach up to 28 days after inoculation. The results suggest that *E. coli* survives for extended periods of time both on leaves and in the soil. This work is critically important for producers and regulatory agencies in developing effective agricultural practices for fresh cut produce.

Radiation sensitivity of fresh vegetables tested. The produce industry is requesting a “kill” step to ensure the microbial safety of fresh produce and to gain the consumer confidence. ARS scientists demonstrated that a moderate (?—kGy isn’t very reader-friendly) dose of 1 radiation can achieve at least a 99.999 percent reduction of *E. coli O157:H7* inoculated onto the surface of fresh produce. ARS further examined the effects of irradiation on the quality of 13 common fresh-cut vegetables (Iceberg, Romaine, red and green leaf lettuce, spinach, tomato, cilantro, parsley, green onion, carrot, broccoli, red cabbage, and celery) and found that the appearance, texture, and aroma of most of the vegetables were not negatively affected, even after 14 days storage, although the vitamin C content was reduced in a few vegetables. Further, no detectable amount of furan (a possible carcinogen) was produced from irradiation. This information is critical to the real-world application and implementation of irradiation as a food safety intervention for fresh produce.

Blade tenderization evaluated as food safety concern. Blade tenderization is a process whereby needles are used to tenderize whole muscle pieces of meat that are then cut into steaks, but may force cells of pathogenic bacteria residing on the outside of the meat into its center. The question remained, however, whether cooking would be adequate to kill cells that are inside in addition to those on the surface of the steaks. ARS scientists evaluated whether cooking blade-tenderized steaks on a commercial gas grill would eliminate *E. coli O157:H7*. In cooking the steaks on an open-flame gas grill to internal temperatures ranging from 120° to 140°F, scientists discovered that regardless of steak temperature or thickness, 1,000 cells of the pathogen could be readily killed. These results validate that mechanical blade tenderization transfers *E. coli O157:H7* into the interior of steaks, with the majority of the cells remaining in the top 1 cm of the meat, but that by cooking the blade-tenderized steak on a commercial-style gas grill, any pathogen cells distributed throughout the meat can be effectively eliminated. This information is critical for regulatory agencies such as the USDA-Food Safety Inspection Service Program, industry, and consumers.

Detection of anthelmintic drug residues enhanced. The monitoring of veterinary drug residues in meat and milk products is a critical issue for regulatory agencies world-wide. ARS scientists developed and validated a new liquid chromatographic-tandem mass spectrometric (LC-MS/MS) multi-residue method to simultaneously quantify and identify 38 of the most widely used anthelmintic veterinary drugs (including benzimidazoles, macrocyclic lactones, and flukicides) in milk and liver. The procedure utilizes a simple modification of the ARS-developed QuEChERS method, which was initially developed for pesticide residue analysis. The new method is capable of detecting and quantifying sufficiently low levels of all targeted drug residues, and was successfully validated for implementation in regulatory monitoring labs in the United States, European Union, and other countries.

Antibiotic resistance in Salmonella studied. Antibiotic resistant bacterialpathogens pose a serious threat to human and animal health. The bacterial strains that are resistant to multiple antibiotics (MR) frequently harbor plasmids (extra-chromosomal circular DNA) or other mobile genetic elements carrying resistance genes that can be spread to non-resistant bacteria, and it is necessary to assess the prevalence of such elements and understand the mechanisms of transmission. ARS scientists examined 4 small plasmids conferring kanamycin resistance in *Salmonella* strains that cause human illness, determining that these plasmids are closely related to each other and to other plasmids from *E. coli* and other pathogens that cause gastrointestinal illness and that they exist in moderately high abundance within bacterial cells. Moreover, since their presence is not routinely assessed by typing methods used to characterize the MR plasmids, the prevalence of this plasmid group may be underestimated. This study underscores the need for regulatory and public health agencies to continue to survey the emergence and spread of MR strains and to monitor the presence of this group of plasmids conferring resistance to kanamycin in *Salmonella*.

Detection of other pathogenic *Escherichia coli*. Serogroups of *E. coli* such as O2, O63, O28ac are of serious public health concern, and improved methods for their detection and identification are needed. ARS scientists developed multiplex PCR (polymerase chain reaction) assays that target genes specific to each serogroup based on DNA sequence information. Research results showed that these genes could be used as diagnostic markers to rapidly identify these serogroups in food and other types of samples.

Shiga toxin-producing *E. coli* (STEC) from swine evaluated for pathogenicity. STEC from cattle are known to cause disease in humans; however, it was unknown whether swine harbored similar pathogens. ARS scientists isolated and examined over 200 different STEC strains from swine feces to determine the presence of genes involved in causing disease (virulence), resistance to antibiotics, and acid tolerance. Results indicated that the strains possessed 22 different combinations of virulence genes, including genes found in *E. coli* strains that cause disease in humans and animals, displayed resistance to a number of antibiotics (with one strain resistant to 15), and were also found to be resistant to acids; acid resistance is particularly critical, given its vital role in the survival of bacteria in acidic foods and in determining the dose needed to infect humans. The study clearly demonstrated that swine STEC are a heterogeneous group of organisms possessing important virulence genes that can potentially cause human illness, information that is critical for producers and regulatory agencies to implement safe and effective production practices.

Optical pathogen detection method created. Rapid methods for the detection of harmful bacterial pathogens in food are necessary to prevent the distribution of contaminated foods, thus protecting consumers from bacterial food poisoning. In collaboration with Purdue University, ARS developed a semi-automated Bacteria Rapid Detection using Optical scattering Technology, or BARDOT system, to identify bacteria from colonies on a plate without the need for expensive and time consuming biochemical or microbiological tests. The simple system involves growing the pathogens from the food in specialized liquid culture media, subsequent plating on a solid media, and then shining a red laser light through a bacterial colony and collecting the image generated using a digital camera. Under test conditions, the actual BARDOT analysis took 5-10 minutes to identify all bacterial colonies, illustrating the system’s utility as a simple and rapid method with direct application to the food industry and regulatory agencies. The BARDOT system (now patented) has been validated for the identification of *L. monocytogenes*, *E. coli*, *Salmonella,* and *Vibrio* isolated from food and for *L. monocytogenes* isolated from clinical samples. Three BARDOT systems are currently being built for collaborative evaluation by an academic, a government, and a food testing reference lab.

On-farm risk factors for *Yersinia enterocolitica* assessed. Swine are the major animal reservoir for *Y. enterocolitica* strains, a major human foodborne pathogen and one of the eight bacterial foodborne pathogens under FoodNet surveillance. ARS scientists examined critical factors for *Y. enterocolitica* infection based on their screening of feces and tonsilar swabs of hogs on 122 premises. Four risk factors were identified (location, E. coli vaccination, deaths due to scours [diarrhea], and diet components) and the odds ratio for infection determined. This study was the first attempt to identify risk factors for *Y. enterocolitica* in the United States hog population, and its results are critical for swine producers and regulatory agencies such as the USDA Animal and Plant Health Inspection Service (APHIS).

Aflatoxin contamination of almonds prevented. Aflatoxin contamination is a critical issue for the California almond industry, valued at over $2 billion annually. ARS scientists found that the navel orange worm (NOW), an insect pest, is a major contributor to alflatoxin contamination of almonds, carrying spores of the *Aspergillus* fungus to the almond kernel through feeding wounds produced by the insect, where the spores subsequently germinate and grow. This finding will have an immediate impact on the almond industry by facilitating the implementation of insect control measures using host-plant volatiles (HPVs), which trap, confuse, or distract the insects from locating the host-plant.

Bioavailability of botulinum neurotoxins in mice poison evaluated. Along with potent neurotoxins (BoNT), the crude food poisoning mixture produced by *Clostridium botulinum* bacteria includes other non-toxic proteins. ARS scientists compared the toxicity of crude versus purified BoNT preparations presented to mice in food and showed that these accessory proteins protect BoNT from destruction and deactivation in the digestive tract. These results will help scientists understand how these potent biothreat toxins work and develop strategies for maintaining a safe and secure food supply.

Risk factors for produce contamination studied. Little is known about specific plant factors that affect the colonization of lettuce by the human pathogens *E. coli O157:H7* and *Salmonella enterica*, and such information is necessary in order to develop adequate good agricultural practices (GAPs) and Hazard analysis critical control point (HACCP) guidelines for the produce industry. ARS scientists demonstrated that enteric pathogens had higher growth rates and grew to greater population sizes on the young inner leaves than on the older middle leaves of Romaine lettuce, a difference partly attributable to differences in nitrogen levels available to bacterial cells on the surface of the leaves. These observations are valuable for risk assessment of produce contamination, the development of efficient sampling strategies to detect contamination of produce, and the design of a control strategy by modulating nitrogen levels in lettuce fields.

*Campylobacter* outbreak from milk contamination assessed. The second largest outbreak of *Campylobacter* illness in U.S. history occurred as a result of contaminated milk processed at a California correctional facility dairy. At about the same time, a small outbreak of *Campylobacter jejuni* illnesses occurred due to the consumption of raw milk from a small organic dairy. A study of both dairies by ARS scientists in Albany, California, in coordination with the California Department of Public Health, involved sampling cattle feces, water, milk, and other samples and isolating and screening *C. jejuni* using a novel method. Suspect outbreak-related strains were genotyped and compared to the human outbreak strains. The successful isolation of outbreak-related strains from both dairies provided crucial epidemiological information for explaining the outbreaks, and the results obtained were critical for ongoing negotiations between producers and State of California representatives concerning coliform levels in raw milk deemed acceptable for human consumption.

Genetic markers identified to assess fungal population biology. The use of atoxigenic strains of *Aspergillus flavus* as biocontrol agents has been discussed due to the potential development of “super” toxin producers. To assess this possibility, genetic markers are required to measure the potential for genetic exchange. In order to determine genetic relationships among various atoxigenic and toxigenic strains of *A. flavus* and to determine the rate at which genes are exchanged among the various strains, ARS scientists developed molecular markers and identified 68 microsatellite loci from the genome sequence of *A. flavus NRRL3357.* Among these microsatellite loci, 24 sites were identified as useful for population biology studies on genetic distribution across strains, variability within locations, and lack of interfering variation in adjacent DNA locations. This work will allow through careful genetic manipulation the design and development of safer biocontrol atoxigenic strains of *A. flavus* that cannot become super toxin producers.

*Escherichia coli O157:H7* higher in cattle fed wet distillers grains. Demand for corn has driven cattle producers to rely on other available feedstuffs, such as wet distillers grains with solubles (WDGS). Small studies on the prevalence of *E. coli O157:H7* in WDGS used in cattle diets have produced mixed results. In the first long-term study on this topic with a large animal group, ARS scientists, utilizing 600 calf-fed steers in the feedlot environment, examined the level and prevalence of *E. coli O157:H7* on hides and in feces for 245 days through the growing and finishing phases of production. They found that feeding 14 percent WDGS (on dry matter basis) in the growing phase was associated with a slightly higher prevalence of *E. coli O157:H7* in the feces compared to animals not fed WDGS, and that in the finishing phase, animals fed 40 percent WDGS had greater prevalence of the pathogen on hides and in feces compared to those not fed WDGS. Thus, the higher prevalence of *E. coli O157:H7* in cattle fed high levels of WDGS could result in a greater pathogen load at time of slaughter.

Heat stress, tolerance, and *E. coli* prevalence studied. Numerous environmental stressors have been identified that may impact infection, prevalence, or levels of pathogens such as *Escherichia coli O157:H7* that are shed by livestock. Over a 2-year period, ARS scientists individually examined feedlot cattle for signs of heat stress on days when the temperature/humidity index was in the “high danger” or “emergency” categories. In addition, the scientists assessed the scoring temperament of individual animals during the normal 28-day weighing schedule to determine stress tolerance. Scientists examined any correlations between heat stress and stress tolerance and *generic* or *E.coli O157* levels and found no relationship between these factors. This information is critical for industry and regulatory agencies in providing healthy cattle at slaughter.

Chemicals identified for destruction of estrogens in water. Worldwide, surface waters are being made more estrogenic as food animals in concentrated confinement secrete natural estrogens and humans secrete synthetic versions of the hormone. Further, both synthetic and natural estrogens may be incompletely removed by municipal sewage treatment plants and agricultural waste handling systems, posing unknown health implications to consumers of the water. In this study, ARS scientists demonstrated that trace levels of Fe-TAML (a commercially available chemical) makes hydrogen peroxide more efficient at degrading natural and synthetic reproductive hormones present in water, demonstrating the chemicals’ potential utility for removing the hormones from agricultural and municipal waste streams. The work will have both national (EPA) and international impact on the bioremediation of water sources.

Drug-resistant *E. coli* in swine. Bacteria have become increasingly resistant to antibiotics, raising concern for health caregivers and the medical community that this resistance may be transferred from animals to humans. Definitive evidence of whether or not this transfer occurs is lacking, however, largely due to the lack of controlled study populations. ARS scientists, in collaboration with Texas A&M University, studied a semi-closed, fully integrated swine production operation and discovered that certain production groups of swine, such as boars and nursery piglets, and certain worker populations, such as slaughter plant workers, were at higher risk of carrying multidrug-resistant *E. coli* than were other populations within the operation. The researchers thus found that certain production practices and occupational exposures may indeed increase the risk of antibiotic resistance transfer in pathogenic microorganisms. The research also identified potential critical control points that may be targeted to reduce the transmission of antimicrobial-resistant bacteria from farm animals to humans demonstrating important food safety implications.

Mechanical vector discovered for *Salmonella* in poultry. The lesser mealworm is a common insect that infests commercial poultry houses. While the mealworm does not overtly affect the birds, it is not known what role the insect might play in the spread of pathogenic or food-poisoning microorganisms within the poultry environment, particularly its capability to infect birds with these microbes. After externally disinfecting the beetle, ARS scientists showed that relatively short exposures of the beetles to low levels of *Salmonella* result in the rapid acquisition of viable bacteria into its alimentary canal, illustrating the beetle’s has potentially a signifcant role as a vector for the pathogen. This work will be extended by project scientists to develop biosecurity procedures against the beetle, which should ultimately result in less pathogen colonization of the birds and, ultimately, in microbiologically safer poultry meat products reaching the consumer. This insect is also being evaluated as a model system for understanding the mechanisms of antimicrobial resistance.

Non-aflatoxigenic *Aspergillus flavus* strains patented. Aflatoxins are a group of toxins produced by the fungus *A. flavus,* which frequently contaminate corn in the mid south area, especially in the Mississippi Delta. Reducing mycotoxin contamination in corn will produce a safer feed/food supply that will be readily acceptable on the world market. ARS scientists have patented a non-toxigenic *A. flavus* strain (K49) that was shown to reduce aflatoxin in corn by up to 93 percent under field conditions. This technology provides the groundwork for industry to test the use of non-toxigenic strains and develop a commercial product that controls aflatoxin contamination in corn.

Antioxidants shown to inhibit aflatoxin production. ARS scientists in Albany, California, and New Orleans, Louisiana, have discovered how to prevent aflatoxin production with safe, common natural chemicals. Caffeic acid, an antioxidant, was found to reduce more than 95 percent of aflatoxin production by *Aspergillus flavus* without affecting fungal growth. In conducting a microarray analysis of *A. flavus* treated with caffeic acid, scientists found decreased expression of almost all genes in the aflatoxin biosynthetic cluster, suggesting that the caffeic acid triggers the induction of alkyl hydroperoxide reductases (enzymes), which prevent the fungus from synthesizing aflatoxin. In addition, the research has elucidated the mechanism by which these compounds cause the fungus to turn off the machinery that synthesizes aflatoxin, which is produced by the fungi to protect it from chemical attacks by plants. This information provides insight into controlling the genes that trigger aflatoxin biosynthesis and should help in devising crop breeding methods to prevent aflatoxin contamination.

Serotyping *Salmonella*. Serotyping *Salmonella* is an expensive, time consuming task; therefore, development of a rapid high-throughput molecular technique is needed by regulatory agencies. ARS scientists adapted a previously developed multiplex PCR *Salmonella* serotyping technique to perform on a high-throughput platform. The new technology can identify the top 31 serotypes, representing 75 percent of all clinically isolated *Salmonella* from humans and animals. This technology significantly improves the efficiency and effectiveness of analysis, allowing the determination of up to 90 isolates in one day at just $1.50/sample, as compared to several days and ~$40.00 for traditional serotyping. Moreover, the technology requires little training, no specific anti-sera, and only standard DNA sequencing instruments. This technique could replace traditional serotyping for most *Salmonella* isolates implicated in foodborne outbreaks. It is currently being tested by several Federal and State public health laboratories in the United States and also by the Public Health Agency of Canada.

Multiplication of *Salmonella enteritidis* studied in eggs. Although chickens infected with *Salmonella* do not deposit this pathogen inside egg yolks very often, there are occasions where bacteria from the surrounding albumen might penetrate through the membrane surrounding the yolk, resulting in rapid and extensive *Salmonella* growth in the nutrient-rich interior contents of the yolk prior to egg refrigeration. ARS scientists used a laboratory egg contamination model to assess the ability of *S. enteritidis* strains to multiply on the vitelline membrane or to penetrate this membrane and multiply inside yolks during incubation at warm temperatures. Studies determined that *S. enteritidis* were all able to penetrate from the exterior of the yolk (vitelline) membrane into the yolk contents during as little as 12 hours of incubation at 30°C and that the concentration of *S. enteritidis* after incubation was significantly higher in whole yolks than in yolk contents at both 12 hours and 36 hours. These results demonstrate that extensive bacterial multiplication on the yolk membrane may occur in addition to (and before) penetration into the yolk contents, further supporting regulatory rules that emphasize rapid refrigeration of eggs for protecting consumers from egg-borne illnesses by *Salmonella*.

Micro-crack detection method developed for table eggs. The USDA Agricultural Marketing Service (AMS) asked ARS to develop a method to help graders identify hairline micro-cracks in table eggs. ARS scientists developed a 20-egg batch-process imaging system to visually enhance and enable detection of these small cracks, resulting in an extremely accurate method to detect the cracks. Further enhancements to the system include a user-friendly, touch-screen database method for recording the number of egg cracks and other egg features that cause downgrades, which the AMS graders are currently documenting. The system will help the graders by increasing their accuracy, removing subjectivity, reducing data transfer errors, increasing their productivity, and dramatically changing the way eggs are currently graded.