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Executive Summary

Research Highlights

Preharvest Pathogen Reduction

Risk of Toxoplasma from retail meat is low The risk of infection with Toxoplasma gondii to consumers resulting from consumption of meats was unknown. ARS scientists in Beltsville MD completed a National Retail Meat Survey for T. gondii in collaboration with the CDC. All 28 planned areas were surveyed, and viable *T. gondii* tissue cysts were found to be less than 0.5 percent in available pork in retail meats. None of the beef and chicken meat samples had live *T. gondii* parasites, based on the bioassays conducted. The National Retail Meats Survey is the first study directly linking pathogen prevalence in animals to consumer risk at the retail meat case. The ARS scientists also found that injection of pork loins with curing solutions containing sodium chloride, and/or potassium or sodium lactate, prevented transmission of *T. gondii*, which is a further safeguard for many pork products.

Calves do not harbor species of Cryptosporidium that are infectious to humans after they are weaned. ARS scientists compared data from feces collected from nearly 1500 dairy calves and found 85% of Cryptospordium-infected pre-weaned calves harbored *Cryptosporidium parvum*, a species highly infectious and pathogenic for humans and other animals whereas 99% of Cryptosporidium-infected, post-weaned calves were infected with species of Cryptosporidium that were neither pathogenic nor zoonotic. This finding emphasizes that special handling of neonatal calves and their manure can prevent or reduce transmission of pathogenic Cryptosporidium to humans, whereas post-weaned calves and their manure pose significantly less risk. The information could influence state and federal regulations regarding farm management, manure management, and proximity of livestock to surface water.

Low risk of pathogens found in raw milk ARS scientists in Beltsville MD analyszed milk samples from the NAHMS 2002 Dairy Survey with a commercial PCR kit for the detection of Salmonella. Their results indicated that Salmonella contamination of US bulk milk is much higher than previously determined using standard culture techniques. While culture techniques indicated that 2.6% (22 /861) of the samples were contaminated, PCR methods indicated that 11.8% (101/854) contained Salmonella. Although the initial levels of Salmonella in the milk were determined to be very low, the presence of this organism represents a risk to consumers of raw milk and/or their products. PCR methods allow for more rapid testing of raw milk, providing results in 24 h as opposed to 48 to 96h for conventional culture methods. In another dairy investigated a large percentage of the cows were excreting Salmonella in their feces even though they showed no disease symptoms. Although Salmonella was frequently detected in the milk filter, it was rarely detected in the milk, indicating that the milking hygiene practiced on the farm was effective in limiting the transmission of high numbers of Salmonella to the milk

The stress response alters salmonella biology In collaboration with researchers at NADC, ARS scientists in West Lafayette IN discovered that Salmonella from swine can grow in an acid environment after they have been exposed to the hormone norepinephrine (NE). This is the first evidence to suggest that transportation stress in swine can actually

enhance the virulence of salmonella infection, and thus increase the opportunity of post-harvest contamination. Following this work ARS scientists in Ames Iowa investigated the prevalence of Salmonella enterica serovar Typhimurium in swine following inoculation with S. Typhimurium exposed to NE in vitro. In the assay to determine the effect of NE exposure on Salmonella survival in the porcine stomach, NE treatment greatly enhanced the survival of the organism. Thus a Salmonella-infected pig stressed during transportation/ mixing can shed NE-stimulated Salmonella and expose naive, stress-compromised penmates with a "primed" microorganism. A complete understanding of this phenomenon will allow the development of strategies to decrease the amount of Salmonella found on swine carcasses at slaughter.

Early vaccination of the neonatal calf can produce strong antigen specific immune responses comparable to vaccinated adult dairy cattle. ARS scientists demonstrated that neonatal calves vaccinated during the first week of life can produce strong and sustained antigen specific, cell-mediated immune responses similar to adult cattle. In vivo and in vitro antibody responses of the vaccinated calf, however, were weaker than corresponding responses of adults suggesting the neonatal calf may be limited in its capacity to mount a humoral (i.e., antibody-based) immune response. To determine if the differing humoral responses of the calf were specific, newborn calves were vaccinated with a foreign antigen, ovalbumin. Results indicated that the calf is capable of robust antibody responses to vaccination. Conceivably, maternal antibody acquired through the ingestion of colostrum may inhibit humoral responses to natural antigens. Infectious diseases of calves are a major economic concern for the U.S. cattle industry, and they also impact human health and food. Morbidity and mortality of preweaned calves costs the industry $90-$180 million annually.

Antiprotozoal screening assay identifies non-drug substances which will kill rumen protozoa. ARS scientists in Ames IA developed an in vitro assay using live/dead fluorescent dyes to screen test compounds for their ability to kill rumen protozoa. Since engulfment of bacterial pathogens by rumen protozoa increases their virulence, i.e. S. typhinurium DT104, strategic defaunation will be advantageous to the dairy and cattle industry fro both animal productivity and health. In the search for rumen defaunation compounds, some natural plant extracts, yucca and rosmary, were found which will effectively control protozoa in the rumen without harming the general fermentation. Rumen protozoa were also inhibited and killed by sodium chlorate, previously shown to reduce the numbers of food borne bacterial pathogens in the rumen. A combination of these two treatments when strategically administered to cattle could greatly reduce the incidence and shedding of salmonella and other pathogens at critical time points in the production cycle of both beef and dairy cattle. Chlorate is being developed as a feed additive because it inhibits the growth of pathogens such as E. coli O157:H7 and Salmonella in live animals. ARS scientists in Fargo ND have determined that the residues of chlorate fall below the safe tissue concentrations estimated by the FDA's Center for Veterinary Medicine which should help speed the regulatory approvals.

The rectal anal junction is a prominent site of E. coli O157:H7 colonization in cattle.

Determining sites where E. coli O157:H7 bacteria colonize and persist in cattle is critical for designing and testing interventions aimed at reducing colonization and shedding. Lymphoid follicle-dense mucosa at the terminal rectum has been identified as an important site of O157:H7 colonization in cattle. ARS scientists in Ames IA and a visiting scientist from Germany studied E. coli O157:H7 binding in tissues and observed O157:H7 bacteria tightly attached to squamous epithelia cells of the rectal anal junction. These findings confirm that the rectal anal junction is a prominent site of O157:H7 colonization in cattle and extend previous studies by showing that these organisms bind to squamous epithelial cells at this site and cause lesions which are similar to the attaching and effacing lesion produced by O157:H7 on enterocytes.

Differing effects of transportation and marketing on Campylobacter and Salmonella in turkeys. Viscera of birds (crops, duodenum, jejunum, ileum, colon, ceca) from six commercial flocks in the Midwest were examined by ARS scientists in Ames IA, in collaboration with university scientists, for *Campylobacter* before and after transport to the abattoir. When data obtained after transport for the six farms was combined, recovery was significantly higher from the gall bladder and crop when compared to on-farm levels. Overall, *C. coli* was isolated more often from the crop and cecum, whereas *C. jejuni* predominated in the intestine (duodenum, ileum, colon) resulting in its more frequent isolation from cloacal swabs. In contrast, with Salmonella no statistical difference was found between the overall prevalence found on-farm and at slaughter, based on any sample type analyzed (crop, ceca, liver/gall bladder, and spleen) with cecal contents having the highest relative sensitivity. This lack of change in prevalence of Salmonella following transportation contrasts with what has been previously reported for swine, and makes it possible to estimate on-farm prevalence in turkeys based on slaughter samples.

Carbodox, a commonly used antimicrobial for swine has varied and "collateral effects": ARS scientists in Ames Iowa discovered that the quinoxaline antibiotic, carbadox, a common antimicrobial fed to swine, stimulates a gene transfer mechanism (a bacteriophage-like gene transfer agent) of the spirochete *B. hyodysenteriae*, the etiologic agent of swine dysentery. The gene mechanism transferred resistance to the macrolide antibiotic tylosin. The carbadox concentrations that stimulate gene transfer are lower than those inhibiting bacterial growth and are equivalent to subtherapeutic in vivo levels. We hypothesize that carbadox can stimulate the transfer of tylosin resistance between *B. hyodysenteriae* strains in the absence of tylosin selection; and further that the "carbadox effect" is not limited to spirochetes. At higher dietary levelsof carbodox in swine diets ARS scientists in College Station TX found that shedding of Salmonella spp in feces was not significantly affected by any dietary carbodox treatment, but carbadox + copper sulfate significantly reduced shedding of Campylobacter spp. in feces. However, coliform and generic E. coli counts were nearly 10-fold higher from animals fed carbadox + copper sulfate. Overall, animal gain tended to be lower in animals persistently shedding Campylobacter spp. during the experiment.

Improved detection method for Mycobacterium avium subsp. paratuberculosis (MAP)

This bacterial pathogen, responsible for Johne's disease in cows and sheep, and possibly implicated in human Crohn's disease, is extremely slow-growing and thus difficult to detect via routine laboratory culture methods. ARS scientists in Albany CA developed real-time PCR and immunomagnetic separation for MAP. Combining these techniques provides unprecedented speed and sensitivity in detection of the bacteria. This method will be useful for future laboratory studies of MAP.

STEC O157 solated from various fair environments following STEC O157 human outbreaks. ARS scientists at Clay Center NE collaborated with County and State Health Departments, State Departments of Agriculture and the CDC in the investigation of human outbreaks of STEC O157 at fairs and petting zoos in Raleigh, North Carolina and Tampa, Orlando and Plant City, Florida. Using methodology they developed in house these scientists were able to isolate STEC O157 from these various fair environments and help to determine the likely outbreak vehicle and sources. In collaboration with North Carolina Department of Agriculture these scientists determined STEC O157 long-term survival and environmental decontamination trial at a heavily, naturally-contaminated agricultural site that had been associated with a large human outbreak. Data suggested that STEC O157 can survive for many months in agricultural soils. In addition, the data suggested that environmental decontamination techniques against STEC O157 will probably not be 100% effective and that some methods have the potential to worsen rather than alleviate environmental STEC O157 contamination.

Candidate genes selected to develop pathogen-resistant lines of poultry. Candidate genes in chickens that are related to host resistance to pathogenic microorganisms were identified by ARS scientists in College Station Texas. These scientists with university collaborators used sophisticated molecular techniques to identify candidate genes that are activated during the avian innate immune response to Salmonella infections. This accomplishment provides essential information both for the selection of new pathogen-resistant lines of poultry and for new poultry treatment therapies for management of food safety pathogens.

Drug-Resistant bacteria do not move freely across human/swine environments
Isolates of the pathogenic bacterium *Enterococcus faecium* that are resistant to the drug vancomycin were found in human wastewater by ARS scientists at College Station, Texas, but not in a swine facility operated in close proximity. Vancomycin-resistant E. faecium (VRE) is of great concern to public health officials, and it is important to know if livestock production operations may be linked to occurrences of VRE. These scientists documented the presence of more than 20 VRE genetic types in human wastewater samples associated with the integrated, semi-closed swine operation; however, VRE were not found in the swine population itself or in wastewater from the swine operation. Thus VRE may occur in humans in other than hospital settings, but there is no evidence that the drug-resistant pathogen moves freely between human and swine populations which is good news for the swine industry.

Turkeys Selected for Fast Growth are More Susceptible to Disease
In turkeys, the association between fast growth and decreased disease resistance has

come primarily through the study of four closed genetic turkey lines developed by at The Ohio State University. Using the cooperator's turkey lines, ARS investigators at Fayetteville, AR, showed that this difference in disease resistance can be attributed to changes in the stress response of fast-growing turkeys as measured by the heterophil/lymphocyte ratio in models utilizing transport stress or dexamethasone treatment. This finding helps to explain the increases seen in turkey osteomyelitis complex since 1988, and will help improve food safety by increasing resistance of turkeys to opportunistic human pathogens.

Humidity is highly important in the horizontal transmission of Campylobacter jejuni
The influence of relative humidity (Rh) on transmission rates is an important factor influencing horizontal transmission of poultry food safety pathogens. ARS scientists at Athens GA placed day of hatch chicks (n = 100 per group) on wood shavings in high (approximately 80%) and low humidity(approximately 30%) controlled pens and challenged with them with C. jejuni using seeder birds. Significant differences in Campylobacter colonization rates were observed between chickens raised under the high and low Rh conditions. A delay in colonization occurred in birds raised under the low Rh conditions which increased with time between removal of birds and placement of newly hatched chicks. This information regarding the importance of humidity could lead to practical applications to help reduce Campylobacter colonization in broilers.

Mechanisms of resistance characterized to extended spectrum beta-lactams in Salmonella from animal sources. Recently, the numbers of Salmonella isolates resistant to the third generation cephalosporins have increased. To investigate the increase in resistance, ARS scientists in Athens GA examined a diverse group of Salmonella serotypes resistant to ceftiofur. The majority of these strains contained the CMY-2 gene and carried large plasmids and integrons often associated with antibiotic resistance. The positive Salmonella serotypes Newport, Heidelberg, or Typhimurium were those most often identified with resistance. These data are important for identifying plasmids which may have increased capacity for transmission and persistence among Salmonella serotypes and is useful for studying mechanisms of resistance among bacterial species.

High degree of genetic relatedness of Enterococcus isolated from retail food items. In a study of retail food (meat, vegetables, and fruit) collected from grocery stores in NE Georgia, genetic profiles of enterococcal isolates were created using BOX PCR and PFGE to determine if genetically related organisms were present. In a few cases, 100% homology in banding patterns was found between isolates from different stores, food types, and in some cases, species. This data from ARS scientists in Athens GA strongly suggest that genetically related enterococci are disseminated among various retail food types in different grocery stores in NE Georgia possibly from a commons source. This work will be useful to scientists involved in Enterococcus research as well as regulatory agencies and the industry and they develop and implement mitigation strategies.

Colonization of reproductive organs and egg contamination by *Salmonella heidelberg*
ARS scientists in Athens GA determined that four *S. heidelberg* strains were all able to colonize the intestinal tracts and invade to reach internal organs of inoculated hens.

Also, all four *S. heidelberg* strains were recovered from the interior liquid contents of eggs laid by infected hens (although less often than an S. enteritidis strain). Eggs containing S. enteritidis in their edible liquid contents have long been known to transmit disease to humans, but recent CDC reports have also linked S. heidelberg infections to eggs. This research demonstrated that some *S. heidelberg* strains can colonize the reproductive tracts of laying hens and cause egg contamination in a manner similar to *S. enteritidis*.

Multiple Salmonella strains can penetrate into egg yolks ARS scientists in Athens GA determined that all tested Salmonella strains were capable of penetrating through the vitelline membrane to reach the yolk contents in 10% to 25% of experimentally contaminated eggs, but variants that were obtained by isolation from infected chickens penetrated through yolk membranes at significantly higher frequencies than did their original parent strains. Although chickens infected with Salmonella do not deposit this pathogen inside egg yolks very often, bacteria from the albumen might penetrate through the membrane that surrounds the yolk, resulting in rapid and extensive Salmonella growth in the nutrient-rich interior contents of the yolk. These results support the need for prompt refrigeration of eggs to minimize the risk that Salmonella will grow to higher (and more dangerous) levels after penetrating into the yolks of contaminated eggs.

Salmonella subpopulations help each other complete the infection pathway to the egg. ARS scientists in Athens GA characterized growth for different genetically defined phenotypes of S. enteritidis subpopulations in over 1900 different environmental conditions. DNA-DNA microarray, ribotyping, and Phenotype Microarray™ were all used to analyze 3 strains of S. enteritidis that varied in phage type and/or growth and virulence properties These results help explain why mixtures of strains have been more reliable for producing contaminated eggs in experimental hen models than using single strains, because subpopulations appear to help each other complete the infection pathway to the egg. This work supports the theory that small evolutionary events contribute to genetic divergence within *S. enteritidis* that aids adaptive radiation into the egg supply.

Postharvest Pathogen Reduction

Intervention strategies for fresh-cut produce. Scientists at Beltsville, MD identified a safe and effective new sanitizer (acidified sodium chlorite, or SANOVA) that achieved a 5-log reduction of E. coli 0157:H7, Listeria monocytogenes, and Salmonella serovar poona on produce even in the presence of large organic loads. The researchers optimized sanitation treatment procedures to ensure good quality of shredded carrot and fresh-cut lettuce while maintaining the effective killing power of the sanitizer. These findings are especially useful to the fresh produce industry as they provide practical information in selecting a suitable sanitizer to maintain microbial safety and quality of fruits and vegetables.

Detecting staphylococcal toxins. A rapid method for detecting staphylococcal enterotoxins is both a critical food safety and food security need. Scientists at Wyndmoor, PA developed a fluorescent latex particle immunoassay (FLPIA) assay with

detection of staphylococcal enterotoxin below 1 ng/mL. This assay has high throughput capabilities where analysis of 50 samples can be completed in 3 hours.

Reliability of cooking thermometers. The USDA-Food Safety and Inspection Service (FSIS) is concerned that consumers cook meat products to safe end-point temperatures before consumption. At the request of FSIS, ARS scientists at Beltsville, MD evaluated the accuracy and reliability of temperature indicator devices used by consumers. The thermometers selected were: digital probe, bimetal probe, forks/tongs, remote wireless, as well as disposable indicators that change color at specified temperatures. None of the thermometers tested consistently reached the end point temperature within the manufacturer's recommended time and several models did not reach the end point temperature even after an extended time period. At the manufacturer's recommended time, the remote wireless thermometers were the least precise and had the greatest variability. The precision and variability of the other categories of thermometers were dependent on meat product and the cooking method. Because the thermometers indicated that the temperature was lower than the actual temperature, consumers using these thermometers when cooking meat products would actually cook the product to higher temperatures ensuring food safety but may reduce the quality of the product. The FSIS will utilize this research to revise their food safety information on usage of instant read thermometers for meat product preparation to further reduce foodborne illnesses.

Portable Assay for Escherichia coli O157:H7. Most illness from E. coli O157:H7 has been associated with eating of undercooked, contaminated ground beef. There is an urgent need for sensitive, specific, rapid detection of these bacteria. ARS scientists at Wyndmoor, PA developed a new assay based on a commercially-available, portable fiber optic biosensor. This assay is specific for E. coli O157:H7 and can detect very low levels (1 CFU/g) of the bacteria in ground beef within 5 hours. Higher levels of contamination can be detected in even less time. The biosensor and battery pack can be carried in a briefcase, allowing assays to be performed at the farm, processing plant, distribution center, and retail store. This capability provides the food industry and regulatory agencies a new screening tool to detect foodborne pathogens and food security threats.

Ready-to-eat (RTE) foods and Listeria monocytogenes. Predicting the behavior of Listeria monocytogenes is a high priority for the FDA and FSIS risk assessment for L. monocytogenes in RTE foods. ARS scientist at Wyndmoor, PA produced robust models that now allow risk assessors and food safety managers to predict the behavior of L. monocytogenes in delicatessen salads at different storage temperatures and product formulations, and commercially prepared Queso Blanco cheese. The models improve the accuracy of the Federal risk assessment for L. monocytogenes, provide science-based information for regulatory policy, and assist food companies in designing salad formulations that present lower health risks to consumers. The research also helped US food companies meet new Federal regulations.

Methods to detect Vibrios in seafood. In collaboration with the Haskins Shellfish Research Laboratory, Rutgers University, ARS scientists at Dover, DE developed and validated a new, rapid, and inexpensive, enzyme-based assay (COPP) to detect pathogenic Vibrionaceae in seawater and shellfish. The assay may be used in identifying

peak periods when Vibrionaceae are at their highest levels in East, West, and Gulf Coast oysters and growing waters. The assay may also prove to have utility as a predictive index and could be used to regulate shellfish harvesting based on Vibrionaceae levels, much like fecal coliform levels are currently used to regulate harvesting waters for fecal pollution. Since the test does not require sophisticated or expensive equipment it may also find use to screen water quality in aquaculture facilities to forewarn the producer or processor of potential problems so that remedial actions may be initiated. There may also be clinical applications for the assay in quickly screening cultures for the presence of Vibrionaceae.

Development of an assay for viruses. Viruses are responsible for a large percentage of foodborne illness in the United States. ARS scientist at Dover, DE developed a real-time molecular method to quickly and easily detect a broad spectrum of Noroviruses, and hepatitis A and E viruses in the stools of infected individuals. This method involves the detection of viral RNA by real-time reverse transcription - polymerase chain reaction (RT-PCR). The assay for example, allows over 90% of the strains of NVs circulating in the world today to be detected within 3 hours in a single tube. Viruses are not only a problem in water and foods such as shellfish, but are commonly associated with outbreaks on cruise ships, Navy vessels, and among troops, particularly during Operation Desert Storm. The technology will have immediate application in both the regulatory and clinical setting.

Decontamination of melons. Outbreaks of foodborne illness due to consumption of fresh-cut cantaloupe contaminated with bacterial pathogens continues to be a concern to regulatory agency's and industry. ARS scientists at Wyndmoor, PA developed new washing procedure and sanitizer treatments for whole and fresh cut cantaloupe. Hot water surface pasteurization with water at 76 C for 3 min using commercial-scale equipment resulted in reduction of Escherichia coli and Salmonella population in excess of 99.999% (5 logs). Experimental and simulation data on thermal penetration profiles indicated that the internal temperature of melons treated with hot water did not increase rapidly compared to the rind temperature. Edible flesh 10 mm from the surface of the rind remained cool regardless of the process temperature. The data obtained clearly demonstrate the efficacy and utility of this treatment for reducing the risk of foodborne illness from melon consumption, while maintaining sensory qualities and extending the shelf life of fresh-cut cantaloupes. Cut melons pieces could also be directly treated with nisin plus sodium lactate or sodium lactate plus potassium sorbate to effectively reduce pathogen populations without adverse effects on quality attributes.

Effects of irradiating foods. Furan is a toxicant and a potential human carcinogen. A recent survey by the Food and Drug Administration (FDA) revealed furan is present in a wide variety of foods that undergo heat treatment. ARS scientists at Wyndmoor, PA investigate possible furan formation in fruit juices as a result of ionizing radiation, a non-thermal processing technology used for enhancement of food safety and extension of shelf-life. The results showed that irradiation and thermal treatments induced furan in both apple and orange juices. The furan levels induced by irradiation at doses that are sufficient to inactivate 99.999% of common forborne pathogens were comparable to

those found in the FDA survey. Additionally, several groups opposed to food irradiation have maintained that 2-dodecylcycloutanone (2-DCB), a chemical found at trace levels only in irradiated foods, is mutagenic and that irradiated foods are therefore carcinogenic. To address this issue 2-DCB was tested for its ability to cause mutations in human cells. The chemical did not cause mutations in human cells (TK6 lymphoblasts). Results of these studies assist federal regulatory agencies to make science-based decisions derived from factual information on the toxicological safety of irradiated foods. For example by the USDA-Food Nutrition Service School Lunch Program in order to provide school district administrators and parents with accurate science on irradiated ground beef.

New purification/concentration method for noroviruses. Norwalk virus is the causal agent of 67% of food-borne illness cases in the US and is believed to cause the highest incidence of food-borne illness linked to fresh produce. Because the infectious dose for Norwalk virus is low and the virus cannot be cultured, a highly sensitive method is crucial for its detection in foods. Scientists at Albany, CA developed a method using magnetic beads to concentrate the virus in contaminated samples, and perform reverse-transcriptase polymerase chain reaction (RT-PCR) to detect the virus with minimal interference from RT-PCR inhibitors present in the samples. This new method will have great impact for surveillance and epidemiological investigation by public health agencies, and the technology may be used by the industry that develops detection kits for application in the food industry and the health care sector.

Genome sequencing and source-tracking. Sequencing microbial genomes yields fundamental information about the organism and is critical for definitive knowledge about pathogens. ARS scientists from Albany, CA in collaboration with The Institute for Genomics Research (TIGR) sequenced the genomes of four different species of Campylobacter. The sequence data revealed new information regarding the population structure, virulence factors, lateral transfer of DNA, gene regulation, and metabolism of Campylobacter species. The Albany scientists subsequently developed a new genotyping system used to genotype >500 strains of C. coli isolated from a variety of sources including humans, animals and food. A strong association between animal host and sequence type was identified and indicated potential biological fitness differences among C. coli strains. Genotyping and source-tracking facilitate attribution of human illnesses to animals and possibly foods and epidemiology of outbreaks.

Pathogen detection in complex food samples. Detection of specific pathogens in complex foods is very difficult, expensive and time consuming. Mass spectrometry (MS) can potentially provide a sensitive and rapid method for analyzing microbes. ARS scientists at Albany, CA characterized over 300 Campylobacter strains using by Time of Flight-MS and identified biomarker ions, which are small proteins with nucleotide polymorphisms associated to specific species and sub-species. The technology which can be used for any pathogen of concern including Escherichia coli O157:H7 and Salmonella, provides a fast, high-throughput method for identifying and differentiating specific species and strains, and among many colonies isolated from complex food samples.

Beef hide decontamination. ARS scientists at Clay Center, NE, have previously shown

that effective interventions that reduce hide contamination also reduce subsequent carcass contamination. The hides of cattle are the source of Escherichia coli O157:H7 that contaminates beef carcasses during commercial beef processing. The scientists furthered their work by identifying the most effective reagents to decontaminate beef hides and then evaluating their application in a novel hide washing cabinet system. Sodium hydroxide wash and a chlorinated water rinse were found to provide the best decontamination. When the hide wash cabinet was evaluated using these compounds, hides were cleaner, but more importantly, the contamination of carcasses was significantly reduced. The prevalence of E. coli O157 on pre-evisceration carcasses was reduced from 17% to 2% when the cabinet was in use. As a result of this work, Cargill Meat Solutions, with the strong support of industry and Federal regulatory agencies has installed hide washing cabinets in all of their processing beef plants.

Alternate interventions for beef carcasses. Decontamination of beef carcasses is a critical issue for industry and regulatory agencies. Washing and steam pasteurization technologies have proven effective but are expensive and can use copious quantities of water. Thus alternate methods are needed that offer the same efficacy. ARS scientist at Clay Center, NE at the request of the National Cattlemans Association and the American Meat Institute evaluated the use of low-dose, low-penetration electron beam (E-beam) irradiation for potential use to kill bacteria on beef carcasses during processing. The objectives were to assess how well E-beam irradiation can reduce levels of Escherichia coli O157:H7 on a large beef surface and to evaluate the effect of the treatment on the taste, smell, and color of the product. A low dose of E-beam irradiation reduced E. coli O157:H7, inoculated onto sections of beef, by 99.99% (4 logs). In assessing for effects on beef quality, the flank steak was used as the model muscle and was treated with 5, 10, 25, 50, and 75% penetrating irradiation. None of the flank steak sensory attributes were affected by any penetration treatment. Ground beef formulations consisting of 100, 50, 25, 10, 5, and 0% irradiated beef were tested. A trained sensory panel did not detect any difference between the untreated and either the 5 or 10% treatments. These results suggest that if chilled carcasses were subjected to low-dose E-beam irradiation, aroma and flavor of ground beef would not be impacted. The data presented here show that low-dose, low-penetration E-beam irradiation has great potential for use as an antimicrobial intervention on beef carcasses during processing and minimally impacts the quality of the treated beef products.

Residue detection on poultry carcasses. Pressure from regulatory agencies has required development of more sensitive methods for testing anti-bacterial treatments on raw poultry. New methods are important because poultry processing plants are using a variety of antimicrobial treatments to meet the pathogen performance standards. The use of paired half carcasses as the control and treatment was found to be a more valid microbiological comparison than using different whole carcasses. This method increases efficiency of testing by about 44% for the same amount of lab work. The new method is currently being used by industry to evaluate the microbiological impact of novel antimicrobial treatments.

Transportation of poultry. Campylobacter is the leading cause of bacterial foodborne

illness. Transport coops can serve as a vehicle for transfer of Campylobacter to chickens as they are carried to the processing plant. ARS scientists at Athens, GA completed a study to measure the effectiveness of a low pressure tap water spray followed by an extended dry time to decontaminate the soiled broiler transport coop flooring. The data show that allowing Campylobacter positive gut contents to dry on the floor dramatically lowers the numbers that can be recovered. These data suggest that a coop re-design and implementation by industry could allow very effective decontamination procedures with minimal use of water.

Shell egg washing. Current guidelines on the washing of shell eggs in the U.S. requires that wash water be at least 90F or 20F warmer than the warmest egg entering the processing facility. The utilization of high wash water temperature will cause the egg to become warmer during processing lending to more ideal conditions for microbial growth. ARS scientists at Athens, GA collaborated with scientists at Auburn University to examine the effects of cool water washing of shell eggs on the microbial and physical quality of the final product. The research showed that shell eggs can be processed in the commercial setting at a cooler temperature, which enhances product quality. Furthermore, it was determined that processing facilities equipped with typical shell egg washing equipment would be able to maintain the cooler wash water temperature during processing. A commercial transfer study was conducted in two separate shell egg processing facilities showing the efficacy of the processing change.

Continuous microwave sterilization and aseptic packaging. Sweet potatoes are an under-utilized commodity. Expansion of the market for sweet potato puree similar to pumpkin puree would provide farmers with a market for 40% of the sweet potato crop, which currently is left in the field because of small size or poor shape that makes them unsuitable for sale. ARS researcher at Raleigh, NC developed an aseptically packaged vegetable puree, processed by a continuous-flow using a microwave heating system. A patent application has been filed. Further studies are in progress to validate the process for microbial safety, as well as to expand its application to other fruit and vegetable purees. This technology provides a new process to convert sweet potatoes and other highly nutritious fruits and vegetables into shelf-stable functional ingredients suitable for use in a variety of formulated food products.

Mycotoxins

Development of a *F. verticillioides* microarray enhances study of the regulation of fumonisin biosynthesis and the fungal interactions with corn. To better understand the genetic pathways that regulate fumonisin production in *F. verticillioides* and to identify genes that contribute to the ability of this fungus to infect corn and cause ear rot, ARS scientists in Peoria IL developed a *F. verticillioides* microarray in collaboration with the Institute for Genomic Research (TIGR). This microarray which contains >11,000 F. verticillioides gene sequences can monitor the expression of all 11,000 gene sequences simultaneously while previous technology allowed for analysis of expression of only one gene at a time. Microarray analysis will enhance study of the regulation of fumonisin biosynthesis and the interactions of *F. verticillioides* and corn which should in turn aid in the development of strategies to control the presence of fumonisins in U.S. corn.

Rapid method developed for moniliformin Moniliformin is a mycotoxin produced by several fungi that are pathogenic to cereal grains. It is acutely toxic to both animals and plants, and is found in maize, wheat, rye, rice, and oats worldwide. Scientists in Peoria IL developed a sensitive, reproducible, and reliable analytical method to separate and quantify moniliformin and applied it to samples of field-inoculated maize. The method involves the separation of the moniliformin using a strong electrical field (capillary electrophoresis). The method is more rapid than traditional liquid chromatographic methods, and may find use in measurement of this mycotoxin in maize.

Incorporation of new insect resistance genes into corn promises to reduce mycotoxins Insect damage and associated ear mold toxins cause hundreds of millions of dollars in losses each year. ARS scientists in Peoria IL evaluated corn plants that expressed a novel plant-derived gene that produces a protein type previously demonstrated to kill insects. Percent mortality of the representative caterpillar pest was significantly correlated with expression of the gene. These scientists also evaluated hybrid transgenic plants in a model plant system that could express two plant-derived genes known to independently enhance resistance to insects. The plants that expressed both the proteins had significantly less damage by caterpillar and beetle pests, and in some cases significantly enhanced mortality. Incorporation of these genes into corn through multigenic transgenic means should result in reduced levels of mycotoxins, thereby improving the health of animals and people, and increasing the exportability of U.S. corn.

The pear ester kairomone surpasses pheromone-based monitoring for codling moth (CM) pest management. ARS scientists in Albany CA assessed CM-caused nut damage (which leads to aflatoxin contamination) through three types of surveys (nut/fruit drop, canopy counts and harvest) and then correlated results to trap capture rates and flight patterns. These scientists discovered the strong attraction of neonate, newly-hatched larvae to the CM kairomone; and are now conducting field trials to evaluate micro-encapsulated formulations that combine minor amounts of the kairomone attractant and reduced amounts of an insecticide to walnut trees to kill newly-hatched CM larvae.

Aflatoxin control in pistachios with non aflatoxin producing strain.of *A. flavus* The

biocontrol agent, AF36 (a strain of Aspergillus flavus that cannot produce aflatoxins and now in use in commercial cotton fields to reduce aflatoxin contamination), was applied by ARS scientists from Albany CA in a research pistachio orchard. The strain AF36 which occurs naturally in CA, became the dominant A. flavus strain in areas where a single application of this strain was made to the orchard floor. In addition, the strain AF36 survived at high levels in another research pistachio orchard even though it had been more than two years since the strain was first applied. Thus the use of the biocontrol agent AF36 in commercial pistachio orchards in California should effectively reduce aflatoxin contamination of pistachio nuts.

Biocontrol with atoxigenic strains, a process known to be highly effective against the S strain of *Aspergillus flavus*, may be particularly beneficial in South Texas. ARS scientists based in Tucson AZ with university collaborators completed a multi-year study on the distribution of aflatoxin producing fungi in South Texas agricultural fields. The results show that both soil type and crop rotations influence fungal community structure, with high clay soils and cotton rotations favoring incidence of the high aflatoxin producing S strain of A. flavus. Aflatoxin contamination of commercial cottonseed was found to be highest where the S strain was most common and corn production was found to favor higher quantities of A.flavus than either sorghum or cotton production. Thus, strategies, including altered crop rotations, to limit contamination in South Texas should be directed at the frequently overlooked and misidentified S strain of *A. flavus.*

Genes required for fungal infection of crops and toxin synthesis are identified.
To elucidate the genes that govern mycotoxin synthesis by fungi, ARS scientists in New Orleans LA and academic cooperators robotically picked a total of 50,000 expressed sequence tags (EST's) clones and shipped them to The Institute for Genomics Research TIGR for sequencing. So far, over 26,000 cDNA clones of *Aspergillus flavus* have been sequenced and over 22,000 usable DNA sequences have been obtained. A total of 7214 unique genes, ranging from 500 to 1,500 base pair in length, have resulted after annotation and assembly of these EST sequences. These genes can be divided into four categories based on putative function: a) aflatoxin pathway genes; b) virulence/ pathogenicity (disease causing) genes; c) fungal development/sporulation (fungal seed formation) genes; and d) regulatory/signal transduction genes. Further investigations on the functions of these genes by gene knockout experiments are underway which will help to identify genes in *A. flavus* involved in toxin production, disease and field survival.

Genes identified for use in selective breeding of aflatoxin-resistant corn Proteomics were used to identify in corn fungal resistance related and stress responsive proteins/genes in resistant lines selected by ARS scientists in New Orleans LA and/or their cooperators in Mississippi, Georgia, and IITA, Ibadan, Nigeria, for use in marker-assisted breeding through gene based and protein/antibody probe based methods. Genes encoding many of these resistance associated proteins (RAPs) have been cloned, and two from Mississippi (Mp) lines, glyoxalase (GLX) and PR-10, have been further characterized. Corn lines bred at IITA in Nigeria from crosses between U.S. and IITA resistant lines and selected for superior agronomic traits, ear rot resistance and aflatoxin-resistance (in either temperate or tropical backgrounds) for 7-8 generations are now undergoing final testing

for aflatoxin-resistance. These lines will also be field tested for aflatoxin-resistance a final time at IITA. This rate of progress could lead to an official release of resistant lines by early 2006.

No shelled lots of peanuts treated with Afla-guard® tested above the tolerance for peanuts, > 15 ppb of aflatoxin Under the supervision of ARS scientists in Dawson GA, Afla-guard® was commercially produced and applied to approximately 5000 acres of peanuts in Georgia and Alabama for the first time to control aflatoxin contamination. Analyses were run on both farmers' stock and shelled stock peanuts to determine the efficacy of Afla-guard® in reducing aflatoxin. Results showed that treating peanuts with the biopesticide reduced aflatoxin by an average of 85% in farmers' stock peanuts. The reduction of aflatoxin in shelled edible grade peanuts ranged from 69 to 98%. No shelled lots of peanuts that had been treated with Afla-guard® tested at > 15 ppb of aflatoxin, the tolerance for peanuts to be sold for edible product. This compares with one warehouse where 48.4% of the shelled lots from untreated peanuts tested at > 15 ppb of aflatoxin, meaning those lots must undergo further processing with concomitant expense before they are safe for human consumption.

Natural genetic variation to Fumonisin B1 toxicity found in corn lines. ARS scientists in Athens GA demonstrated that fumonisin B1 (FB1) phytotoxin produced by *Fusarium verticillioides*, has adverse developmental affects on certain corn lines. In addition the cellular mechanism of toxicity was shown to be similar to that described in animals, namely disruption of ceramide synthase with the resulting accumulation of sphingoid bases and their 1-phosphates. Since corn lines were clearly either sensitive or resistant to the FB1, natural genetic variation in these lines as it relates to FB1 toxicity is established. This information will enhance future directions for corn breeding programs based on fumonisin resistance, as opposed to the already established fungal-induced disease basis.

Neural tube defects (NTD) found in outbred mice strains commonly used in teratology as well as in highly susceptible inbred strains. ARS scientists in Athens GA demonstrated that fumonisin B1 causes NTD in the CD1 strain of mouse when given according to established protocols. This establishes that NTDs in mice are not, as previously believed, a unique response of the highly inbred and NTD susceptible LM/Bc strain; rather, that a robust, outbred strain commonly used in teratology and toxicology studies is also vulnerable. Results from feeding studies indicate that the no effect level for NTDs in both strains is likely > 50 ppm, that NTDs only occur at maternally toxic doses, and that fumonisins cross the placenta of LM/Bc mice at doses at which no transplacental transfer of fumonisins occurs in CD1 mice--suggesting the placenta is plays an important in NTD susceptibility. These findings will improve our understanding of NTD induction by fumonisins and decrease uncertainty in regard to risk assessment and thus will protect the US corn industry from overregulation due to flawed hazard identification.

Chemical Residues

Immunoassay developed for polybrominated diphenyl ethers Polybrominated diphenyl

ethers (PBDEs) are persistent environmental contaminants that can accumulate through the food chain, but rapid inexpensive methods of detection are not available. ARS scientists in Fargo ND generated antibodies and incorporated them into a rapid screening assay (magnetic particle immunoassay) with the collaboration of Abraxis, LLC. The assay is successful in detecting PBDEs in fish, water, and soil samples with high specificity and sensitivity. The cost and turn-around time for this new assay is significantly less than that of the traditional PBDE analysis; and, therefore, its use may result in affordable, widespread monitoring of these persistent pollutants.

Estradiol and estrone elute from soil columns filled with animal manure When the natural female hormone, estradiol, which is found in animal manures, was applied to soil-filled columns, ARS scientists in Fargo ND found that estradiol and estrone (estadiol degradation product) were the predominant compounds found to elute from the column. As the organic content of the soil increased, more of the estradiol was absorbed to the soil, and the time for complete estrogen-soil binding also increased. Data from a constructed wetland system indicate that wetlands are useful in reducing both N and estrogenic activity from animal waste.

Quantitation and confirmation of antibiotic residues in shrimp. Rapid antibiotic residue detection methods for imported seafood are a critical need. Scientists at Wyndmoor, PA in collaboration with CIAD, Mexico modified and validated a liquid chromatography-fluorescence-mass spectrometry method originally developed by ARS for meat samples, for new application in seafood. The method will find particular application and use for efforts in monitoring imported shrimp for fluoroquinolones residues.

Automated direct sampling and testing. Effective and efficient analytical methods are needed to detect pesticide residues in produce however, maintenance of the detection instrument is a major limitation for routine analyses. Scientists at Wyndmoor, PA developed a novel approach called automated direct sample introduction (DSI) that can eliminate the need for frequent instrument maintenance while also improving detectability for pesticides in complex food matrices. Regulatory laboratories that implement this approach will benefit by improved analytical performance, ease of use, combined with significant cost savings.