Preharvest Food Safety Keeping

STEPHEN AUSMUS (D2152-5)

Left to right: Visiting Spanish veterinarian scientist Sandra Diaz and physiologist Annie Donoghue examine Petri dishes for pathogens like *Campylobacter, Salmonella,* and *E. coli,* while postdoctoral fellow Ixchel Reyes-Herrera and University of Arkansas microbiologist Pamela Blore prepare plates to study the efficacy of natural compounds against pathogenic gastrointestinal bacteria from poultry.

The food you eat every day travels a long way from farm to fork, and dangers—in the form of foodborne pathogens or other contaminants—lurk along that road, waiting to hitch a ride on a lettuce leaf or a piece of beef or chicken. Making sure our food is safe to eat is of paramount importance to Agricultural Research Service scientists across the country. ARS research on food safety is multifaceted and wide ranging. The following touches on some of the agency's research on *Escherichia coli, Salmonella,* and *Campylobacter* and chemical residues in meat.

Lesser Known "Big Six" *E. coli* Targeted in Gene-Based Research

While *E. coli* O157:H7 is perhaps the best known of the *E. coli* species that cause foodborne illness, its lesser known relatives are increasingly of concern. Food safety regulators, public health officials, and food producers in the United States and abroad want to know more about these less-studied pathogens.

That's why ARS scientists such as Pina M. Fratamico and other ARS, university, and corporate coresearchers are developing new techniques to quickly and reliably identify these microbes. The scientists are sorting out who's who among these related pathogens by uncovering telltale clues in the microbes' genetic makeup.

The gene-focused approaches to rapid, reliable, and reproducible detection and identification are paving the way to science-based assays. With further work, the assays might be presented as user-friendly test kits for use by regulatory agencies and others. Foodmakers, for example, might be able to use such kits for in-house quality control, while public health labs might rely on them when processing specimens from patients hospitalized with a foodborne illness. Too, the assays might be used in research to develop a clearer picture of the prevalence of these microbes in food, people, animals, and the environment.

In the past few years, a half-dozen of these emerging *E. coli* species (also called "serogroups") have come to be known among food safety specialists as "the Big Six," namely, *E. coli* O26, O45, O103, O111, O121, and O145.

"These *E. coli* serogroups can produce one or more kinds of Shiga toxin—the compounds that can make us ill," says Fratamico. "We know that some strains belonging to these six serogroups have the potential to cause outbreaks of foodborne illness.

"We also want to develop the PCR-based laboratory assays into field-ready test kits so that we can better understand the prevalence of these strains in food. In addition, we want to determine whether they cause more illness than O157:H7 does, and if so, why.

"These species are virtually indistinguishable from other *E. coli* strains, including nonharmful *E. coli,* when you use conventional culture methods to grow the microbes in the laboratory," says Fratamico. She is a microbiologist and research leader of the ARS Molecular Characterization of Foodborne Pathogens Research Unit at the agency's Eastern Regional Research Center in Wyndmoor, Pennsylvania.

Along with ARS and university collaborators, Fratamico has already developed gene-based PCR (polymerase chain reaction) assays for each of the Big Six. All of these assays are based on unique forms of two genes, *wzx* and *wzy,* that occur in serogroup-specific forms in these microbes. The assays can be performed with either of three widely used PCR options—conventional, real-time, or multiplex. The assays also allow detection of two Shiga toxin genes, *stx1* and *stx2,* so that users can determine whether or not the strain they are scrutinizing is harmful.

Fratamico and coresearchers Connie E. Briggs, Yanhong Liu, Chin-Yi Chen, Xianghe Yan, and Terence P. Strobaugh, Jr., at Wyndmoor; Chitrita DebRoy, Michael A. Davis, and Elisabeth Roberts of Pennsylvania State University-University Park; and Takahisa Miyamoto of Kyushu University, Hakozaki, Japan, are collaborating on this work. Their findings appeared in the following journals: *Applied and Environmental Microbiology, Canadian Journal of Microbiology, Foodborne Pathogens and Disease, Journal of Clinical Microbiology,* and *Molecular and Cellular Probes.*

Pathogens and Chemical Residues Out of Beef and Poultry

STEPHEN AUSMUS (D1465-7)

Microbiologist Jim Wells is investigating the relationship between WDGS-based feed and the incidence and persistence of *E.coli* O157:H7 in cattle manure and on their hides. Here, Wells processes bovine fecal samples for microbial analysis while microbiologist Elaine Berry plates the processed samples for *E. coli* tests.

Of course, microbes like *E. coli* are present in most mammals' digestive tracts. Some ARS researchers are looking into how particular feeds can influence the levels of *E. coli* O157:H7, a particularly problematic strain.

Less Feed Supplement May Mean Less *E. coli*

When corn is converted to ethanol, leftovers from the biorefining process include what are known as "wet distiller's grains with solubles" (WDGS). Typically, they are yellow and have a texture somewhat like that of wet corn meal.

Since 2007, WDGS have been the subject of an array of studies at the ARS Roman L. Hruska U.S. Meat Animal Research Center (USMARC) in Clay Center, Nebraska. The investigations are revealing more details about the pros and cons of adding WDGS to cattle feed. (See "Evaluating an Ethanol Byproduct as a Potential Cattle Feed Ingredient," *Agricultural Research,* September 2009.)

"WDGS are rich in protein and are also a source of energy and minerals," says microbiologist James E. Wells at USMARC. He has led studies to investigate the relation between WDGS-based feed and the incidence and persistence of *E. coli* O157:H7 in cattle manure and on their hides.

Cattle are a natural reservoir for the microbe, which is apparently harmless to them but, of course, can be pathogenic to humans. In addition, *E. coli* in manure can newly infect or reinfect animals in pastures and feedlots. What's more, *E. coli* on hides can contaminate carcasses at the packinghouse.

In early experiments with 608 steers, Wells and coinvestigators at Clay Center provided the animals with either a corn-based feed (corn grain and silage) or a 40-percent WDGS feed during the finishing stage, that is, the last 16 weeks before harvest.

The team's analyses showed that the incidence and prevalence of *E. coli* O157:H7 in manure and the incidence on hides was significantly higher for the WDGS-fed cattle than their corn-fed counterparts.

"The differences may be due to changes within the animal's digestive system, such as an increase in gastrointestinal pH, possibly caused by eating the WDGS," says Wells. "But other factors may also have played a role."

The study, one of the largest and most detailed of its kind, was made possible in part by USMARC's well-equipped labs, large research herd—representing many leading cattle breeds—and extensive network of research pens and other facilities that simplify collection of specimens.

Wells, along with research leader Tommy L. Wheeler, Steven D. Shackelford, Elaine D. Berry, Norasak Kalchayanand, and other colleagues at Clay Center, published some of these findings in a 2009 article in the *Journal of Food Protection.* The research was funded in part by the Beef Checkoff, a promotion and research program financed by U.S. beef producers.

Additional studies are planned. "We're still not entirely certain why feeding 40 percent WDGS resulted in higher levels of *E. coli* in cattle manure," says Wells. "There are economic and performance benefits to feeding this ethanol coproduct, so we need to find ways to reduce the *E. coli* O157:H7 effect before we make recommendations about WDGS to producers."

Another tactic to control *E. coli* may come in the way of vaccines for cattle.

New Vaccines: Can They Quell *E. coli* O157: H7 in Cattle?

Though much remains to be discovered about sometimes-deadly *E. coli* O157:H7, most experts readily agree that cows—whether dairy or beef—are a major reservoir of this foodborne pathogen. With that in mind, it's easy to understand why a team of ARS scientists,

Animal caretaker Wally McDonner provides feed supplemented with a yeast extract to Japanese quail to test the feed's efficacy against *Salmonella* and *Campylobacter.*

STEPHEN AUSMUS (D2155-14)

STEPHEN AUSMUS (D2154-4)

Microbiologist Gerry Huff (left) inoculates chicken embryos in tests to determine the virulence of bacteria as technician Dana Bassi uses a digital egg monitor to determine embryo viability.

led by microbiologist Vijay K. Sharma, is creating vaccines designed to undermine the pathogen's undisputed success in colonizing cattle intestines.

The microbe can grow in the bovine digestive tract without causing any apparent harm to the animal. In humans, of course, it's a different story: In us, foodborne *E. coli* O157:H7 can cause severe gastroenteritis, bloody diarrhea, and sometimes life-threatening hemolytic uremic syndrome.

Sharma and ARS microbiologists Thomas A. Casey and Evelyn A. Dean-Nystrom (retired) have developed two experimental vaccines that show promise for disrupting colonization. By so doing, the vaccines would also reduce long-term shedding of the microbe into the animals' manure.

STEPHEN AUSMUS (D2156-1)

In efforts to develop new techniques to quickly and reliably identify pathogenic *E. coli* serogroups, microbiologist Pina Fratamico (left) and molecular biologist Yanhong Liu (center) view real-time PCR results from study samples as microbiologist Lori Bagi loads a thermal cycler with more samples for testing.

Shedding is a normal part of any persistent colonization, according to Sharma, and is vital to the pathogen's spread from one animal to the next and throughout the environment.

Manure-borne *E. coli* O157:H7 poses several hazards. On the ranch or at the feedlot, the microbe can travel, via rainfall, into drinking water or into irrigation water that may later contaminate vegetables or other fresh produce. At the packinghouse, *E. coli* O157:H7 in manure that is stuck to cattle hides or carcasses may end up contaminating equipment or meat.

Sharma and Casey, based at the ARS National Animal Disease Center (NADC) in Ames, Iowa, have tested the vaccines in preliminary experiments with 24 healthy calves. Their research included giving some of the animals a placebo or either of the vaccines, both of which were modified strains of heat-killed *E. coli* O157:H7. In tests of their immunity, calves were exposed to live *E. coli* O157:H7. Among the results: Fifty percent of the calves that received either of the two experimental vaccines stopped shedding *E. coli* O157:H7 within 1 to 2 days after being exposed to the live pathogen. What's more, blood tests taken 28 days after the first vaccination showed that blood levels of antibodies—immune system proteins—against certain *E. coli* O157:H7 colonization proteins were significantly higher in calves immunized with either of the test vaccines.

Vaccines Are Minus One or Two *E. coli* Genes

In creating the vaccines, the scientists deleted either a single *E. coli* O157:H7 gene of interest, *hha,* or two genes, *hha* and *sepB*. These genes affect the ability of the pathogen to produce and secrete proteins known as "LEE," short for "locus of enterocyte effacement." These proteins have an important job: They help *E. coli* stick to intestinal cells.

"LEE-promoted adherence to intestinal cells," says Sharma, "is a prerequisite for successful *E. coli* colonization of cattle, persistence in their intestines, and shedding of the microbe in manure."

What happens when the *hha* or *hha* and *sepB* genes are missing?

"We've shown that exposing calves to heat-killed *E. coli* O157:H7 that's missing one or both of these

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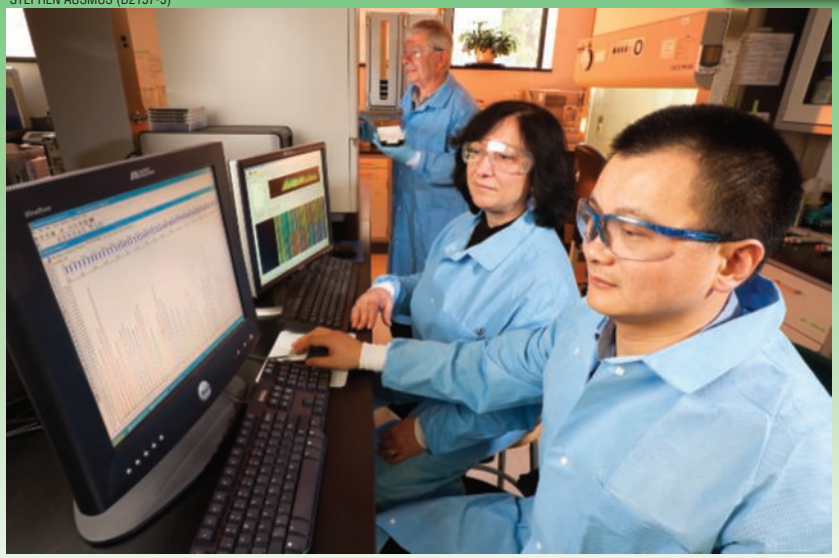

Molecular biologist David Needleman (in the background) loads DNA to be sequenced in an automated DNA sequencer as microbiologist Pina Fratamico (center) and computational biologist Xianghe Yan view sequence data from *E. coli* O145.

genes causes the animals to create a large amount of antibodies against several important LEE proteins," Sharma reports.

His investigations into *hha*'s role in *E. coli* O157:H7 date back more than a decade. His team was the first to isolate and clone *hha* from *E. coli* O157:H7. Now, Sharma and coinvestigators are the first to select *hha* and the *hha-sepB* combination as the basis for experimental vaccines designed to protect cattle from *E. coli* O157:H7 colonization.

Their early studies appeared in the *Journal of Bacteriology* in 2004 and the Federation of European Microbiological Societies' *FEMS Microbiology Letters* in 2005.

The idea of vaccinating cattle against *E. coli* O157:H7 isn't new. Some commercial vaccines have already been developed, for example. But America's cattle are not, at present, routinely vaccinated against the microbe. That may change, especially if effective, affordable, easy-to-prepare and easy-to-use vaccines become readily available. Such vaccines could make food safer for us and could reduce the costs and consequences of outbreaks of foodborne illness traced back to *E. coli* O157:H7 contamination. With further research and testing, the *hha*- and *hha-sepB*-based vaccines may prove ideal for providing such protection.

Organic Poultry's Special Needs

To conduct research that may be beneficial to the organic industry, ARS has a new state-of-the-art organic poultry research facility that was developed collaboratively between an ARS unit in Fayetteville, Arkansas, and the University of Arkansas. The facility not only meets the livestock requirements of the U.S. Department of Agriculture National Organic Program (NOP), but also the animal welfare recommendations for poultry by the National Organic Standards Board and the Organic Poultry Guidance Document of the Accredited Certifiers Association.

In the United States, organic poultry production has increased almost 20 percent annually since the establishment of the NOP in 2002. This program accredits private businesses, organizations, and state agencies to certify producers and handlers of agricultural products according to NOP regulations. (The Fayetteville farm was certified under Nature's International Certification Services.) Organic poultry farms can only use compounds on the national list of substances allowed for organic production. Their use of antibiotics and other drugs and pesticides available to conventional poultry producers is restricted or prohibited. Alternatives to antibiotics are also needed for conventional poultry production, since regulations for antibiotic use are being tightened in response to the prevalence of antibiotic resistance in pathogens.

Microbiologist Gerry Huff at ARS's Poultry Production and Product Safety Research Unit (PPPSRU) in Fayetteville has investigated yeast extracts as alternatives to antibiotics for controlling disease-causing bacteria in turkey poults. Details of the study can be found in a paper published in 2010 in *Poultry Science*.

"Organic, natural remedies and preventatives are particularly needed for organic poultry production," says Huff. "Our lab has been studying

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Technician Dee Kucera (foreground) harvests *E. coli* O157:H7 isolates from agar plates as technician Shannon Ostdiek (left) plates samples for *E. coli* O157:H7 isolation and microbiologist Jim Wells uses a robot to enrich *E. coli* O157:H7 from samples.

the effects of yeast extract as an immune stimulant and alternative to antibiotics in conventional turkeys. Initial studies suggest that dietary yeast extract has good potential as a nonantibiotic alternative for decreasing pathogens in organic turkey production. We need a larger study to confirm its efficacy. But it is expensive to work with turkeys—they eat a whole lot—so we are now using yeast extract in Japanese quail studies to test its efficacy against *Salmonella* and *Campylobacter*. We're using quail as a model system to evaluate natural treatments that will be beneficial for chicken and turkey production."

Huff's current study, in collaboration with Irene Wesley at NADC, involves 800 Japanese quail—a number they couldn't do with turkeys. Yeast extracts help boost the immune system's ability to kill bacteria, but there is a downside.

"Yeast ramps up certain aspects of the immune response, but this can decrease body weight in some individuals," says Huff. "Weight gain is suppressed because the energy normally used for growth is redirected toward the immune system. We need to balance the two effects of adding yeast extracts to turkey feed."

PPPSRU research leader Annie Donoghue is looking at an integrated systems approach to reducing *Salmonella* and *Campylobacter* in organic and all-natural poultry.

Because drugs are not permitted in organic production, mortality may be higher than in conventional poultry operations. "Food safety concerns with *Salmonella* and *Campylobacter* are high-priority areas for organic poultry producers, and strategies that promote gut health, limit disease, and prevent foodborne infections are needed," says Donoghue. Working collaboratively with professors Kumar Venkitanarayanan at the University of Connecticut and Dan Donoghue at the University of Arkansas, she found that caprylic acid, naturally found in milk and coconut oil, has efficacy against these foodborne pathogens when fed to poultry.

These studies were published in *Poultry Science* (January 2009) and the *Journal of Food Protection* (April 2009).—By **Sharon Durham** and **Marcia Wood,** ARS.

This research supports the USDA priority of ensuring food safety and is part of Food Safety, an ARS national program (#108) described at www.nps.ars.usda.gov.

To reach scientists mentioned in this article, contact Sharon Durham, USDA-ARS Information Staff, 5601 Sunnyside Ave., Beltsville, MD 20705-5129; (301) 504-1611, sharon.durham@ars.usda.gov.

ARS and FSIS Take On Chemical Residues

Chemical residues of any kind are of concern in food-producing animals. Steven Lehotay and Marilyn Schneider at the ARS Eastern Regional Research Center (ERRC) in Wyndmoor, Pennsylvania, and colleagues with the USDA Food Safety and Inspection Service (FSIS) in St. Louis, Missouri, are developing both field-based and laboratory-based testing methods to detect veterinary drug residues in cattle.

For screening, the ERRC team and the FSIS Midwestern Laboratory compared the three major in-plant tests in use: the fast antibiotic-screening test (FAST), which was used by FSIS at the time of the study, and recently developed commercial tests called "PremiTest" and "KIS Test" (kidney inhibition swab). All three tests were evaluated in both kidney exudate and blood serum, and the new commercial tests were more effective and faster than FAST. These findings were used by FSIS to help them choose KIS to replace FAST for monitoring antibiotics in kidney tissues from cattle at slaughter establishments.

STEPHEN AUSMUS (D2160-9)

Chemist Steven Lehotay prepares samples for analysis to determine the presence of veterinary drug residues from kidney extracts.

The ARS team used their own instrument-based method to test for 121 drug residues at a time from more than 200 samples from culled dairy cows collected from a slaughter establishment. FSIS is working with the ERRC group to implement the new approach at the FSIS laboratories.

Chemist Janice Huwe, in the Animal Metabolism-Agricultural Chemicals Research Unit in Fargo, North Dakota, teams up with FSIS in an ongoing effort to find out whether unwanted chemicals are in meat animals. Huwe and her colleagues survey domestic food-producing animals from federally inspected slaughterhouses across the country for the presence of chemicals like dioxin and PCBs—toxic environmental pollutants—and PBDEs, flame-retardant chemicals used in electronics, clothing, and household goods.

And there is good news. A comparison of data from the two collection years of 2002 and 2008 showed declining trends for all the pollutants—decreases of up to 25 percent in beef, chicken, and turkey. Pork levels showed no change but remained at levels that were nearly undetectable. PBDE pollutants were reduced by more than 50 percent in each food category. This is most likely because PBDEs were removed from production in the United States in 2004.—By **Rosalie Marion Bliss,** ARS.