E. coli

Unwelcome From Farm to Fork

KEITH WELLER (K9082-14)

Animal physiologist Mohammad Koohmaraie observes technician Julie Dyer as she steam vacuums a section of a beef carcass.

Just the name *E. coli* O157:H7 strikes fear in the hearts of consumers who want to avoid becoming ill from this food pathogen. To ease these fears, Agricultural Research Service scientists continue to study new ways to keep the dangerous bacterial strain out of meats and other foods.

Children and the elderly who eat food tainted with *E. coli* O157:H7 are among those most at risk of serious illness and sometimes death. Symptoms include chills and bloody diarrhea.

Cattle feedlot managers are typically unaware that animals harbor the pathogen because cattle are rarely sickened by the microbe, says William W. Laegreid, who heads the Animal Health Systems Research Unit at the Roman L. Hruska U.S. Meat Animal Research Center (MARC), Clay Center, Nebraska. Until recently, that meant feedlot managers first learned of the bacteria's existence in their operations only when its origins were traced back to them from meat samples at the processing plant. But now MARC scientists have developed methods that detect even a few *E. coli* O157:H7 in manure.

Preliminary results of an ongoing ARS survey of fecal samples from feedlot cattle in nine midwestern states show about 1 percent of the animals harbor the microbe in December through February. But during warmer weather, from July through October, the scientists found the organism in up to 75 percent of the samples—a surprise.

"We had expected to find less than 1 percent of cattle harboring the microbe year-round," said Laegreid.

The survey was possible because of the improved detection methods developed in 1994 by MARC scientists to isolate and culture *E. coli* O157:H7 from manure. But first the scientists had to make highly specific and sensitive monoclonal antibodies (MAbs) for a certain antigen produced by *E. coli* O157:H7. These MAbs have since become part of a simple rapid-testing kit that's now widely used in the food industry and that was FDA approved in August 1999 for testing on human clinical samples.

In a 1997 survey, the MARC researchers found that *E. coli* O157:H7 infection in weaning calves may be at least as prevalent before they reach the feedlot as after.

Improved detection techniques, while showing the problem in greater magnitude than before, offer a reason for hope. The researchers now can more easily see whether ideas for reducing the occurrence of *E. coli* O157:H7 work—and some do.

For example, MARC veterinary medical officer James E. Keen fed hay rather than a finishing ration to market-ready cattle for 7 days. Rapid testing showed that tactic alone reduced the bacteria's prevalence from 52 percent to 18 percent. Likewise, when marketing conditions were simulated, the prevalence dropped from 53 percent to 14 percent. The simulation involved loading cattle onto commercial trailers, hauling them for 2 hours, and holding them overnight with access to drinking water but no feed.

Interventions at processing plants also reduce the incidence of the microbe. One study showed that 28 percent of cattle entering four large midwestern processing plants were actively shedding *E. coli* O157:H7 in their feces. Forty-three percent of 341 carcasses were initially contaminated with the bacterium, as were 11 percent of hide surfaces. But after processing was complete, only 6 of 330 carcasses, or 1.8 percent, showed some contamination, said ARS animal physiologist Mohammad Koohmaraie, who heads the MARC Meats Research Unit.

Searching for *E. coli* Fingerprints

To find where *E. coli* O157:H7 contamination is most likely to occur in a packing plant, MARC scientists employ a method that public health officials use to track the source of bacteria. The procedure, called pulsed-field gel electrophoresis, produces a DNA fingerprint that is unique to a particular strain.

"By using this method we've found that at least 68 percent of the *E. coli* O157:H7 on meat traces back to a live animal of the same group of cattle," says microbiologist Genevieve A. Gallagher.

At five points in the processing plants the researchers sought evidence of *E. coli* contamination, just as plant employees do routinely in a troubleshooting program that complies with standards set by USDA's Food Safety and Inspection Service (FSIS). Such programs are known as Hazard Analysis and Critical Control Point procedures.

The Meats Research Unit helps to fulfill FSIS' information needs, such as developing protocols for microbiological testing or researching steam vacuuming of beef carcasses—a practice approved by FSIS and now used in most meat packing plants.

KEITH WELLER (K9085-1)

Using DNA fingerprinting, microbiologist Genny Gallagher identifies bacterial isolates in carcass-contamination tracking studies.

KEITH WELLER (K9089-1)

Before testing for antibodies to potential foodborne bacteria, veterinarian William Laegreid takes a blood sample from a steer.

"Presently, we are researching the question, 'Have some strains of *E. coli* O157:H7 become well adapted to acids in the gastrointestinal tract of cattle in the feedlot?'" says microbiologist Elaine D. Berry. Here's why the answer is important: In many processing plants, an organic acid-wash solution is used for cleaning carcasses. Acid-resistant strains could render the wash solution ineffective.

A Special Glow

To better assess potential protocols for decontaminating meat and to gain basic insights into how *E. coli* O157:H7 attaches to carcass surfaces, the researchers have genetically engineered the microbe so that it glows. A very sensitive video camera known as an intensified charged-coupled device system, equipped with a 60-millimeter lens, enables the scientists to see for the first time contamination patterns on large areas of carcass surface tissue.

"By studying the attachment as it happens, we don't have to first sample the surface and culture the bacteria to find where they are located," says microbiologist Gregory R. Siragusa, who is now at the ARS Richard B. Russell Research Center's Microbiological Safety Research Unit, Athens, Georgia.

The Leader So Far

The scientists hope to find ways to prevent *E. coli* O157:H7 from binding to the carcass surface because attempts to wash off bound bacteria may be futile. Then, during further processing, contamination may spread.

The scientists recently developed a multistep treatment before meat grinding that holds promise against *E. coli* O157:H7 proliferation. The most effective treatment so far is a multistep process: A high-pressure water wash, followed by a hot water spray, then a hot air treatment, and finally a spray of 2-percent food-grade lactic acid. While the treatment almost imperceptibly darkened the color of refrigerated ground beef patties, it reduced microbe numbers in beef trim by 99.9 percent.

Hunting Harmful Genes

With a tenacity akin to *E. coli* O157:H7's own penchant for posing health concerns, the scientists are striving to determine which genes make the bacteria harmful. They're studying the DNA piece by piece in a procedure called subtractive hybridization. They take DNA from the *E. coli*, remove parts known to be harmless, and then isolate and identify the remaining pieces. The scientists then research the cellular processes encoded by the unique pieces.

For producers, the scientists are working toward breeding farm animals that resist infection with pathogenic *E. coli* strains. Using new techniques, Laegreid and his colleagues have identified cattle genes that are expressed in response to a molecule called a lipopolysaccharide, which is produced by *E. coli* O157:H7.

"Knowing which genes respond to foodborne pathogens is a first step toward developing interventions," says Laegreid. Next, scientists will pinpoint genetic markers that one day may be used to select farm animals that won't harbor the harmful bacteria or help them propagate.—By **Ben Hardin,** ARS.

KEITH WELLER (K9090-1)

Technician Carolyn Johnson uses monoclonal antibodies to confirm *E. coli* O157:H7 presence in cattle fecal samples.

This research is part of Food Safety, an ARS National Program (#108) described on the World Wide Web at http://www.nps.ars.usda.gov/programs/appvs.htm.

Mohammad Koohmaraie and William W. Laegreid are at the USDA-ARS Roman L. Hruska U.S. Meat Animal Research Center, P.O. Box 166, State Spur 18D, Clay Center, NE 68933; phone (402) 762-4100, fax (402) 762-4111, e-mail koohmaraie@email.marc.usda.gov laegreid@email.marc.usda.gov. ◆