Targeting E.coli Infections at Their Source

Getting at bacteria before they have a chance of getting into people is the focus of ARS studies in Ames, Iowa, aimed at stopping a particularly nasty *E. coli*-related disease.

Microbiologist Evelyn Dean-Nystrom and veterinary medical officer William Stoffregen of ARS's National Animal Disease Center (NADC) have worked to pinpoint where *E. coli* O157:H7 bacteria lurk in calves. Nystrom is also working with scientists at the Uniformed Services University of the Health Sciences in Bethesda, Maryland, to develop and test an oral vaccine that eliminates *E. coli* O157:H7 bacteria from cattle.

Nystrom works at NADC's Preharvest Food Safety and Enteric Diseases Research Unit, while Stoffregen works at the center's Bacterial Diseases of Livestock Research Unit.

Enterohemorrhagic *E. coli* O157:H7 is the most common infectious cause of bloody diarrhea in people in the United States. Hemolytic uremic syndrome, a potential consequence of *E. coli* O157:H7 infection, is the primary cause of acute kidney failure in U.S. children.

Where Bacteria Hide

E. coli is normally found in the intestines of all animals, including humans, where it suppresses growth of harmful bacteria. But *E. coli* O157:H7 is a rare variety that produces large quantities of potent Shiga toxins, which can cause severe damage to small blood vessels and kidney tissue.

Undercooked or raw ground beef has been implicated in many *E. coli* O157:H7 disease outbreaks among humans. In the United States, Shiga toxin-producing *E. coli* causes diarrhea in more than 100,000 people each year, with *E. coli* O157:H7 responsible for more than 70,000 of these cases.

"In cattle, these bacteria almost always have no easily discernible effect," says Nystrom. "That's a major reason why *E. coli* O157:H7 is hard to detect in them."

STEPHEN AUSMUS (K11311-18)

At the National Animal Disease Center in Ames, Iowa, visiting scientist Joachim Pohlenz, a pathologist from the School of Veterinary Medicine in Hannover, Germany, selects and trims intestinal tissues to be processed and examined for signs of *E. coli* O157:H7 infection.

Nystrom and Stoffregen found that, in addition to intestines, calves' gall bladders may be good indicators of whether an *E. coli* O157:H7 infection has taken place. The gall bladder stores and secretes bile, which includes salts used to break down food.

The researchers found that signs of bacterial infection were present in the gall bladders of 12 of 13 calves used for their study. Four days after Nystrom and Stoffregen inoculated weaned calves with *E. coli* O157:H7 bacteria, most had developed cholecystitis (inflammation of the gall bladder), and many had lesions and *E. coli* O157:H7 bacteria in their gall bladders.

"This discovery identifies the gall bladder as a possible niche for *E. coli* O157:H7 infection in cattle and as a potential source of Shiga toxin-producing *E. coli* contamination of beef products," says Stoffregen. "Including gall bladders in samples cultured for *E. coli* O157:H7 may help identify infected cattle at slaughter," adds Nystrom.

Intimin Is Vaccine's Key Ingredient

The key ingredient of the oral vaccine—developed at the Bethesda university's Department of Microbiology and Immunology—is intimin, a protein on the outer membrane of the O157:H7 strain. The bacteria need intimin to attach themselves to intestinal tissue.

Nystrom assisted with this study early on by showing that calves injected with purified bacterial intimin would develop antibodies against it. "This confirmed previous studies in mice that showed that intimin-specific responses reduced adherence of *E. coli* O157:H7 bacteria to both cultured tissue cells and to intestinal cells in the intact animal," she says.

Nystrom's work also revealed that intimin-fighting antibodies interfere with *E. coli* O157:H7 colonization and lessen intestinal damage in newborn pigs.

STEPHEN AUSMUS (K11310-19)

Microbiologist Evelyn Nystrom and technician Bryan Wheeler vaccinate a calf orally with intimin-expressing tobacco cells mixed with milk replacer.

Earlier studies found that pregnant pigs vaccinated against bacterial intimin developed antibodies against it in their sera and colostrum. Also, newborn piglets experimentally challenged with a Shiga toxin-negative *E. coli* O157:H7 strain, and who ingested colostrum from intimin-vaccinated pigs, had fewer of the inoculated bacteria in their intestines than did piglets nursed by nonvaccinated pigs.

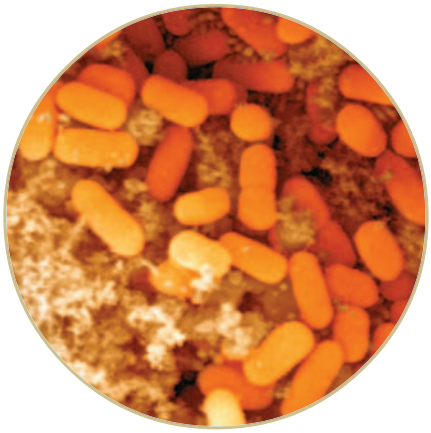
Microbiologist Alison O'Brien, who chairs the university department, is working closely with Nystrom in efforts to prove that the vaccine is effective in cattle and to develop a plant containing the vaccine that cattle will eat. "We want an inexpensive, effective, easily administered vaccine to prevent cattle from becoming infected with *E. coli* O157:H7, thus blocking transmission of these organisms to humans," O'Brien says. Corn is a potential candidate for development into an intimin-producing edible plant for livestock, she adds.

Nystrom says a vaccine directed against intimin will not affect colonization by beneficial, non-disease-causing *E. coli* bacteria because these bacteria do not produce intimin.

The vaccine was developed by Nicole A. Judge, a graduate student in O'Brien's laboratory who transferred the gene that encodes for intimin into a non-nicotine tobacco cell line. Tobacco cells are the standard ones used to determine whether plants can express a foreign antigen. In the lab, the cell line was freeze-dried into powder that was then rehydrated with buffer, mixed with milk, and given to calves.

Nystrom explains that the vaccine as first created could not be produced in sufficient quantities to make enough intimin to be effective in cattle. Wayne Curtis, a Pennsylvania State University professor, was contracted to address this problem. He successfully scaled up production of intimin-expressing tobacco cells so that a vaccine could be effective for calves.

EVELYN NYSTROM (K11302-1)



Scanning electron micrograph of *E. coli* in the gall bladder of an experimentally infected calf. Magnified about 5,000 times.

"*E. coli* O157:H7 is a very dangerous infection, and cattle are an important source of it," says Nystrom. "One way to reduce the risk of infections in humans is to reduce the level of these bacteria at the source point. Through vaccines such as this one and by pinpointing where the bacteria hide in cattle, we can contribute greatly to making beef an even safer consumer product."—By **Luis Pons,** ARS.

This research is part of Food Safety (Animal and Plant Products), an ARS National Program (#304) described on the World Wide Web at www.nps.ars.usda.gov.

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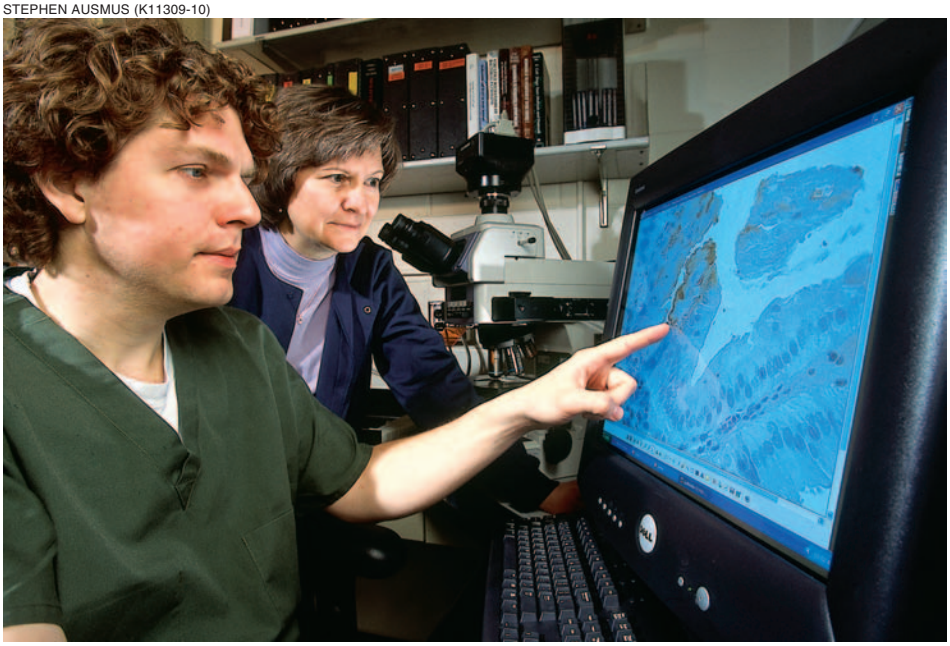
STEPHEN AUSMUS (K11309-10)



Veterinarian Bill Stoffregen shows microbiologist Evelyn Nystrom epithelial surface lesions and brown-stained *E. coli* O157:H7 bacteria in the gall bladder of an infected calf.