

### **Research Update White Paper**

### Background

Research is the foundation on which the industry's safety initiatives have been built. The Beef Industry Safety Summit has become a premier forum to highlight some of the latest advances and their application within industry.

The beef industry, through The Beef Checkoff, has devoted more than \$28 million to beef safety research since 1993, according to Bo Reagan, senior vice president of research, education and innovation for the National Cattlemen's Beef Association (NCBA). That investment has been leveraged with private industry dollars of approximately \$350 million per year in development and implementation of safety practices. During the 2009 Beef Industry Safety Summit, researchers and private industry representatives summarized results from studies that will advance the industry's understanding of beef safety risks, and also highlighted advancements in beef safety interventions.

The Beef Industry Safety Summit is funded in part by the Beef Checkoff and coordinated by the Beef Industry Food Safety Council (BIFSCo) and NCBA, and continues the tradition of industry-driven efforts to improve beef safety. An overview of the entire summit is available in an executive summary that can be accessed at <a href="www.bifsco.org">www.bifsco.org</a>. This report summarizes the research presented in more detail. Additional beef safety research information can also be accessed at <a href="www.beefresearch.org">www.beefresearch.org</a>. Posters for each research summary presentation can be found on both Web sites.

### **Research Summaries**

## Control of Pathogens in Beef: Recent Accomplishments of the ARS Food Safety Program

Steve Kappes, Ph.D. and Molly Kretsch, Ph.D., USDA-Agricultural Research Service summarized recent accomplishments of the ARS Food Safety Program. Projects are conducted primarily at four Agricultural Research Service locations in Nebraska, Iowa, Texas and Pennsylvania. The goal is to improve food safety by controlling pathogens in the animal, and the animal production environment. ARS conducts research on *E. coli* and other foodborne pathogens important in beef through a collaborative and systematic approach. Kappes and Kretsch summarized key research accomplishments in both preharvest and post-harvest beef safety.

This year, a 5-year retrospective review of the ARS National Food Safety Program will be conducted, customer and stakeholder workshops held, and a new strategic plan for the next five years developed. The goal is to address the critical issues regarding beef safety for both regulatory agencies and industry, using the most recent and advanced technologies.

### E. coli O157:H7 vaccine updates

**Jim Sandstrom, DVM, Epitopix** announced that the U.S. Department of Agriculture (USDA) had granted Epitopix a conditional license for a vaccine to reduce *E. coli* O157 prevalence in cattle. The vaccine was developed initially to address *Salmonella* issues in turkeys. Since bacteria require iron for survival, and it is acquired via siderophore and porin proteins

(SRP), the vaccine stimulates immunity against these cell-surface proteins, which in turn reduces iron acquisition. The Salmonella SRP vaccine was approved for use in cattle by USDA in 2004 and approximately five million doses were used in the U.S. dairy industry in 2008. A feedlot efficacy study demonstrated the effectiveness of the vaccine in reducing E. coli O157:H7 prevalence in cattle by 85 percent versus cattle that received a placebo vaccine. The company will be conducting additional efficacy and potency studies in cooperation with industry and Kansas State University to satisfy USDA requirements for a full license. Studies are also underway to determine the efficacy of administering the vaccine to calves prior to their arrival at the feedlot.

Gary Weber, Ph.D., Bioniche presented a summary of research results beginning in 2002 for the development of an E. coli O157 vaccine. The vaccine acts by stimulating cattle's immunity against a certain class of proteins that facilitate bacterial attachment to intestinal cells. A 2007 study conducted by the University of Nebraska and published in the Journal of Food Protection demonstrated that vaccinated feedlot cattle were less likely to be colonized at the terminal rectal mucosa (TRM), the most significant point of *E. coli* O157 attachment. Subsequently, the cattle shed less pathogenic *E. coli* in their feces. A more recent study, also conducted by the University of Nebraska, and published in the peer-reviewed scientific journal Foodborne Pathogens and Disease demonstrated a 92 percent reduction in E. coli O157 colonization in vaccinated cattle. The company is currently working through licensing requirements.

Results of a feedlot study were presented by **David Smith, DVM, Ph.D., University of Nebraska**. The research project examined the effects of an *E. coli* vaccination (Bioniche) on feedlot cattle. The potential impacts of feeding high levels of wet distiller's grains plus solubles on the probability for feedlot cattle to shed *E. coli* O157:H7 in feces was also examined as part of the project. The vaccinated steers were 43 percent less likely to shed *E. coli* O157:H7; however, cattle fed the wet distiller's grain diet (40 percent dry matter basis) were 2.1 times more likely to shed *E. coli* O157:H7 than cattle fed the control diet. Smith emphasized

that more research should be conducted to discover how cattle ration components affect *E. coli* O157:H7 shedding, and the potential to use dietary interventions as a means to control this pathogen.

#### **Pre-harvest interventions**

Pat Mies, Ph.D., Ivy Natural Solutions presented information on two products that the company is in the process of commercializing. Past research has demonstrated the effectiveness of sodium chlorate supplementation to reduce *E. coli* O157:H7 in cattle. Ivy Natural Solutions has been working to license the technology and will perform a technical efficacy study in 2009 based on direction from the Food and Drug Administration (FDA).

The company is also developing a hide spray that incorporates bacteriophages to reduce *E. coli* O157:H7 on the hides of cattle presented for harvest. The spray application was shown to effectively reduce *E. coli* O157:H7 in beef trim by more than 50 percent compared to beef trim from cattle that were sprayed with only water. Certain bacteriophages act by destroying their host cells—in this case *E. coli* O157:H7. Phages have been approved for use by the FDA to reduce *Listeria* prevalence in cheese and ready-to-eat meats.

#### Salmonella and beef safety

David Renter, DVM, Ph.D., Kansas State University provided an overview of two studies examining Salmonella in beef cattle production systems. In one study, the researchers monitored Salmonella in feedlot cattle both at arrival at the feedlot and at harvest. The fecal prevalence was 65 percent and 73.3 percent, respectively. The research group is currently examining whether the Salmonella isolates of food safety concern—those detected on the day of harvest—are genetically similar to the Salmonella present in the cattle when they arrived at the feedlot.

In a second ongoing study, Renter and his colleagues are examining the effectiveness of a *Salmonella* vaccine on the prevalence and serotypes of *Salmonella* in cattle feces throughout the feeding period up to harvest. The vaccine's impact on cattle health and performance is also being evaluated. Approximately 1,600 head of cattle are included in the study.

Guy Loneragan, B.V.Sc., Ph.D., West Texas A&M University summarized the outcomes of studies addressing the burden of methicillin-resistant Staphylococcus aureus in cattle populations and Salmonella in culled dairy cattle. Conducted in collaboration with several other researchers and institutions, samples were collected from 301 animals for the drug resistance phase of the project. Methicillin-resistant Staphylococcus aureus was not recovered from any sample; however, methicillin-susceptible S. aureus was recovered from 14.3 percent of the animals.

For the Salmonella study, fecal samples were collected from 706 cull dairy cows sourced from nine dairies. Salmonella was recovered from 32.6 percent of the cows. The prevalence varied by month of sample collection and by the dairy from which the cows originated. Dairies that practiced whole-herd vaccination against Salmonella had a significantly lower prevalence than herds that did not vaccinate. Of the 667 isolates recovered, 91.9 percent were resistant to only one drug, whereas 3.6 percent were resistant to more than four drugs. Loneragan felt the results of the project demonstrated Salmonella may be commonly recovered from cull dairy cattle in the Texas High Plains region. However, most appear to be of serotypes unrelated to human or animal disease and were broadly susceptible to antimicrobials.

# Beef safety in small processing facilities; Effect of diet on pathogen shedding

Mick Bosilevac, Ph.D., USDA-Meat Animal Research Center presented results from two studies. The first evaluated E. coli O157:H7 and Salmonella in small processing plants. According to Bosilevac, little information is available regarding pathogen prevalence in plants that process less than 1,000 head of cattle per day. The researchers collected samples from 1,995 hides and carcasses at seven small processing plants located across the United States. The overall hide prevalence of *E.* coli O157:H7 and Salmonella was 71 percent and 91 percent, respectively. The prevalence of *E. coli* O157:H7 on pre-evisceration carcasses was 33 percent, and for Salmonella was 58 percent. Bosilevac said levels of pathogens on carcasses were reduced at plants that used a hide-wash intervention. These results were similar to data collected in larger beef processing facilities.

In the second study, the effect of feeding wet distiller's grains with solubles on *E. coli* O157:H7 was conducted by collecting hide and fecal samples from 603 feedlot steers. The average percentage of *E. coli* O157:H7 enumerable hide samples was not significantly different between diets, but the group of calves fed wet distiller's grains with soluble did have higher levels. The results indicated feeding 40 percent wet distiller's grains may increase the level and prevalence of *E. coli* O157:H7 in cattle, but the magnitude of the difference detected in this study may have been affected by the low prevalence in the control cattle.

Dennis Burson, Ph.D., University of Nebraska presented results examining the effect of multiple Escherichia coli O157:H7 antimicrobial interventions in very small beef processing facilities. The researchers visited three very small meat processing facilities and applied three interventions (lactic acid wash prior to evisceration, hot water wash after carcass splitting and trimming, and a final lactic acid wash just prior to chilling). The control treatment consisted of one lactic acid wash at the end of slaughter, just prior to chilling. Reductions in aerobic plate counts were greater for the carcasses that received multiple interventions versus the control carcasses. Preintervention control and experimental treatment carcasses tested 17.3 and 18.6 percent positive for E. coli O157:H7, respectively. After chilling, the control and experimental carcasses tested 2.7 percent and 1.3 percent positive for E. coli O157:H7, respectively.

#### **Antibiotic resistance**

John Sofos, Ph.D., Colorado State University discussed results from a study examining the prevalence of potentially antibiotic-resistant bacteria carrying class 1 integrons in water and fecal samples from farm, urban, and natural environments. Samples for this project were collected from Colorado cattle operations (water before distribution to animals, drinking water, waste water and animal feces) and non-farm sources (lakes, rivers and animal feces within city limits and in the Rocky Mountain National Park). The levels of potentially ceftiofur-resistant bacteria were highest in fecal samples from urban environments and lowest in samples from natural environments, while the lowest levels of potentially

tetracycline-resistant bacteria were observed in fecal samples from an urban environment. Levels of potentially antibiotic-resistant bacteria were similar in all types of water samples. These results contribute to the better understanding of the levels and distribution of antibiotic-resistant bacteria in agricultural and non-agricultural environments.

Dayna Harhay, Ph.D., USDA-Meat Animal Research Center presented information on a project characterizing multi drug-resistant (MDR) Salmonella isolated from cull and fed cattle at slaughter in the United States. Contamination of beef with Salmonella can significantly impact consumers and the beef processing industry. The emergence of MDR Salmonella is an important health problem as drug treatment options may become limited, especially for cases of invasive Salmonellosis.

In this study, the average prevalence of *Salmonella* on hides, pre-evisceration and post-intervention carcasses was found to be 89.6 percent, 50.2 percent and 0.8 percent respectively. The proportion of the *Salmonella* population found to be multi drug-resistant was on average 17.4 percent, 11.8 percent and 0.33 percent on hides, pre-evisceration and post-intervention carcasses respectively. In spite of these differences, multiple hurdle processing interventions were effective and decreased carcass contamination with *Salmonella* on average by 98.4 percent. Hide and carcass levels of MDR *Salmonella* were not significantly different for fed or cull cattle slaughtered at the same facility.

## Analysis of novel chemical compounds for the control of *E. coli* O157:H7

Kendra Nightingale, Ph.D., Colorado State
University discussed a project that identified novel chemical compounds with the potential to control *E. coli* O157:H7 during processing. Small molecules (typically < 500 daltons) have been useful to probe biological functions at the molecular and cellular levels as well as for treating disease. The researchers screened more than 64,000 small molecules (compounds from known bioactive and commercial libraries) for bactericidal activity against *E. coli* O157:H7. Forty-six small molecules demonstrated bactericidal activity against a natural *E. coli* O157:H7 strain from cattle feces.

Known bioactive compounds that inhibited *E. coli* O157:H7 growth predominantly included antimicrobials that have commonly been used as clinical interventions. Further investigation revealed two compounds used in non-clinical applications damage bacterial outer cell layers and alter membrane permeability, suggesting that they will be effective in controlling a broad spectrum of pathogens. The utility of the small molecules identified that inhibit *E. coli* O157:H7 will be examined in future studies as dipping and spraywashing treatments to reduce *E. coli* O157:H7 populations on cattle hides and beef tissues.

For more information, visit

www.bifsco.org www.beefresearch.org

#### or contact

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