REPORT OF THE COMMITTEE ON FOOD AND FEED SAFETY

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The Committee met on November 14, 2010 at the Minneapolis Hilton Hotel in Minneapolis, Minn., from 1:00 p.m. to 5:00 p.m. There were 16 members and 32 guests present. Dr. Lafontaine gave an overview about why it is important for USAHA consider the new information available on the pathogen, *Escheria coli* 0157:H7. He thanked the researchers and other presenters for being so willing to be here at their own expense. Dr. Buntain took lecture notes that are in this report. Presenters provided abstracts or papers that are included in their entirety at the end of the Committee report.

Chemical Sensing in Enterohemorrhagic *E. coli* (EHEC) and Cattle Associations

Vanessa Sperandio, PhD, University of Texas Southwestern Medical Center]

This presentation described the genes necessary for EHEC to colonize cattle. In the rumen, EHEC needs acid resistance capacity genes to make it to the intestines to colonize. EHEC will utilize driver proteins from other bacteria to enable EHEC colonization. Three key genes and AHLs from other bacteria are needed for EHEC to colonize. They found that the AHLs are not present downstream of the rumen (alkaline pH represses AHLs). Bacillus cereus was cloned to inactivate the effect of AHLs. Epidemiological implications are to get AHLs antagonists. Algae have antagonist characteristics to do this. Probiotics is another possibility with lactonase gene to colonize the rumen. Engineering a bacteria is another theory. She believes a combination of approaches to alter the rumen ecosystem may be possible to decrease EHEC colonization. In humans AHL could not be detected in the stomach or gut and thus not published (negative results). Funding is from NIH for the basic molecular studies and not for beyond the proof of principle. The abstract in its entirety is included at the end of this report.

Ecology of *E. coli* 0157:H7 in Cattle: Interactions and Interventions

T.G. Nagaraja, DVM, Kansas State University

There are hundreds of serotypes that produce shiga toxins and these serotypes also contaminate meat. Little is known about these organisms and about 1/3 of illnesses from EHEC are from non-O157:H7 EHECs. All production systems have presence of O157:H7. Organic cattle have similar shedding of O157:57 as conventional and “natural” production systems. Prevalence is from 2-5% to 10-80% of cattle shedding, often intermittent or transient. Lots of animal to animal and location to location variability have been found, with a range from 0 to 100% pen to pen variation and feedlot to feedlot varied from 0-59.4% fecal positive shedding. Flies can amplify its growth. Birds were also shedding O157:H7. Seasonal shedding is typically more May to October, 8-80% and much less in winter. Diets associated with prevalence in their studies show forage feeding to increase shedding. Distiller’s grains feeding if greater than 20% will significantly increase shedding. However, mechanisms remain unknown. The rectum is a preferential area of the gut for colonization in cattle (2010 data). They found that fecal prevalence underestimates the prevalence in the

animal. Thus the hindgut should be a focal point of studies, especially the rectal-anal mucosal junction area which is difficult to swab in the live animal. A carcass contamination CCP is hide pulling that physically translocate or aerosolize EHEC; the other is evisceration contamination of carcass, but hide removal is more important to minimize carcass contamination. Control strategies, which include good farming practices, (Diet? Feed additives, sodium chlorate, Tasco seaweed product, probiotics), pathogen interventions prior to slaughter (bateriophages and hide spraying systems), good processing practices, and proper consumer cooking are all potential interventions at various stages of research. It is a complex issue needing a multiple intervention hurdle approach. The abstract is included at the end of this report.

Super-shedding of Escherichia coli 0157:H7 by Cattle

Terrance Arthur, PhD, USDA-ARS, U.S. Meat Research Center

Cattle hide is the major source of carcass contamination of cattle carcasses (78% versus 1.3% contamination on pre-evisceration carcasses when hide was chemically dehaired). Super shedders (10^4/gram) are responsible for disportional amount of O157:H7 transmission in feces. In pastures about 50% shedding occurs. They found that the prevalence goes up and increases the number of super shedders, especially after 20% on hide prevalence or higher than 200 CFU/g in feces. These guidelines can be used by producers to measure intervention effects. For transport and lairage effects, feedlot interventions can be negated with transportation in unclean, unwashed trucks. Genetic fingerprinting experiments showed that at lairage there are new strains contaminating the carcasses and hide. On the carcass only 15% came from feedlot sources, the rest were from trucks and processing plant. Within 2 hours of being in lairage, the cattle can become contaminated and super shedders can contribute to that. A potential intervention is a hide wash cabinet. This reduces recent contamination if a hide washer can be put prior to the kill floor. No 0157:H7 was found in lymph nodes. Super shedders were found everywhere in the cow GI system. This will increase the risks of cross contamination of final product when a super shedder is processed. Carcass contamination appears to cluster around the super shedders. There are no proven interventions at lairage, to date, that can be absolutely recommended. The abstract is included at the end of this report.

Pre and Post Harvest Interventions- A Processor's Perspective

Daniel Schaefer, MS, Cargill Beef

Cargill's' approach to reduce *E. coli* O157:H7 includes a focus on public health outcomes and to make science-based decisions. Chronically infected a animals can be carriers of pathogens into meat, even intact products. Examples of hide-on carcass wash systems were shown. Pre-evisceration wash was described as a two part water spray and lactic acid mist system. Continuous monitoring of key parameters is critical for trend analysis. Thermal treatments were then described. Acid rinses were shown next. Carcass chilling is used to take quickly reduce the surface temperature to 40 degrees to prevent microbial growth. At that step, carcass mapping is done to validate the system. Sub-primal cabinets with smaller pieces are sprayed by an acid just prior to bagging. SmartHarvest is used as a determination of line speed by measuring criteria that can contribute to potential problems. Video monitoring screens located throughout the system for reviewing animal welfare, slaughter and processing to monitor the human element and reviewed with employees weekly. New technology discussed was pre-harvest intervention with a vaccine that target siderophores. Currently industry has petitioned FSIS to allow carcass irradiation. The abstract is included at the end of this report.

Opportunities to Reduce the Risk of Shedding *E. coli* 0157 by Cattle: Implications for Beef Safety, the Environment and Public Health

Gary Weber, PhD, Bioniche Food Safety-USA

Seasonality of shedding in cattle and contamination of produce during the summer months are followed by a corresponding peak in human illnesses. Watershed contamination has been studied after outbreaks indicating environmental dissemination. A review of published outbreak investigations and *E. coli* O157:H7 research was shared. Vaccination trials were reviewed from bench to cow-side. It was noted that a "herd immunity" effect occurs with vaccinated cattle. A goal is to create cattle herds with a winter-like shedding profile versus a summer high shedding profile. A spin-off may be less environmental, wildlife and produce contamination. The paper in its entirety is included at the end of this report.

Overview of Meat Regulations Regarding *E. coli* 0157:H7

William James, DVM, USDA-FSIS, Office of Field Operations, Washington, DC

Dr. James reviewed human illness data from CDC up to 2009 indicating a decrease in O157:H7, but an increase in non-O157:H7 STECs. FSIS testing of raw ground beef in 2010, to date, of 9565 samples found 0.27% positive. The Federal Meat Inspection Act of 1906 gave USDA the authority to regulate certain animal products. The regulations were covered that declared meat with this pathogen as adulterated, and the history of HACCP regulations was described. New methods for collecting and analyzing samples continually improve. Small establishments are assisted to implement basic controls to address pathogen reduction. Non-O157:H7 STECs in products is currently of interest to the agency, to include, declaring them an adulterant. This is currently under agency review. The abstract is included at the end of this report.

Committee Business Meeting

Chair Dr. Lafontaine called the meeting to order at 4:35pm. Approximately 12 members were present. The Committee charge was reviewed and input was sought. There were no comments. Next, the process of resolution development was explained. No resolutions were submitted by members or another committee to this committee. The Chair asked if there is any other business to bring forward. The Chair was complemented on the program content. He asked for suggestions for topics on the next meeting which were *Salmonella* in animal feed and *Salmonella enteriditis* in shell eggs. The meeting was adjourned at 5:00pm

Chemical signaling in enterohemorrhagic *E. coli* (EHEC) in cattle colonization

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Abstract

Chemical communication mediates signaling between cells. Bacteria also engage in chemical signaling, termed quorum sensing (QS), to coordinate population-wide behavior. The bacterial pathogen enterohemorrhagic *E. coli* (EHEC), responsible for outbreaks of bloody diarrhea worldwide, exploits QS to promote expression of virulence factors in humans. Although EHEC is a human pathogen, it is a member of the gastrointestinal (GI) flora in cattle, the main reservoir for this bacterium. EHEC cattle colonization requires SdiA, a QS transcription factor that uses acyl-homoserine lactones (AHLs), for proper folding and function. EHEC harbors SdiA, but does not produce AHLs, consequently having to sense AHLs produced by other bacterial species. We recently showed that SdiA is necessary for efficient EHEC passage through the bovine GI tract, and show that AHLs are prominent within cattle rumen, but absent from the other sections of the GI tract. EHEC utilizes the locus of enterocyte effacement (LEE) to colonize the recto-anal junction of cattle, and the glutamate decarboxylase (*gad*) system to colonize cows. Transcription of the LEE genes is decreased by rumen AHLs through SdiA, while transcription of the *gad* acid resistant system is increased. It would be expensive for EHEC to express the LEE genes in the rumen where they are not necessary. However, in preparation for the acidic distal stomachs the EHEC *gad* is activated in the rumen. Hence AHL signaling through SdiA aids EHEC in gauging these environments, and modulates gene expression towards adaptation to a commensal life-style in cattle. Inasmuch as EHEC is largely prevalent in cattle herds, interference with SdiA-mediated QS inhibition of cattle colonization could be an alternative to diminish contamination of food products due to cattle shedding of this pathogen.

Ecology of *Escherichia coli* O157:H7 in Cattle: Interactions and Interventions

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Shiga toxin-producing *Escherichia coli* (STEC) are important cause of enteritis in humans, ranging in severity from mild to bloody diarrhea, and in children the condition may progress to hemolytic uremic syndrome (HUS) and even death. Approximately, 500 O serotypes of *E. coli* have been shown to produce Shiga toxin and over 100 of these have been associated with human sporadic and epidemic diarrheal diseases. The most common STEC associated with human disease is *E. coli* O157:H7. Several non-O157 STEC serotypes, such as O26, O45, O103, O111, O121, and O145, have emerged as important causes of enteritis and it is estimated that non-O157 serotypes account for 20 to 50% of STEC infections annually. Most cases of *E. coli* O157 infections in humans are food-borne and foods implicated in transmission of the organism include beef and dairy products, and fruits and vegetables contaminated with cattle feces. Contamination of beef carcasses with *E. coli* O157:H7 occurs during harvest and is associated with both fecal and hide prevalence. *E. coli* O157 is not a significant animal pathogen, except in colostrum-deprived or immune-suppressed neonatal calves and piglets. *E. coli* O157 occurs in many animals but ruminants have the highest prevalence among the food-animal species. The organism colonizes in the gastrointestinal tract and is then shed in the feces. The hindgut is the major site of persistence of *E. coli* O157:H7 and there is evidence that mucosal epithelium proximal to the rectoanal junction may be the site of preferential colonization.

The prevalence of *E. coli* O157 in U.S. cattle is almost ubiquitous and at the herd level the prevalence ranges from 80 to 100% in grazing, dairy and feedlot cattle. However, significant variation in prevalence occurs among individuals or pens of cattle. The level and duration of shedding is highly variable and intermittent, with some animals shedding for a few days only, while others shed for an extended period, up to a year or longer. Factors that have been shown to influence prevalence and duration of fecal shedding include season and diets. The prevalence of shedding typically increases during summer months (late spring to early fall) and is lowest in the winter. Dietary influences, including grain type and processing method, forage level and quality, and distiller's grains have been associated with fecal prevalence. Specific mechanisms responsible for seasonal or dietary influences have not been understood.

Control strategies to reduce food borne illnesses associated with *E. coli* O157:H7 include good husbandry practices in the farm, pathogen reduction strategies applied at preharvest and postharvest phases and consumer education in handling and cooking of the meat. Implementation of effective preharvest interventions should enhance the effectiveness of postharvest interventions and also reduce environmental contamination with cattle waste, thereby further lower the risk of human food borne or water borne illness. Concurrent use of multiple strategies could synergistically decrease reduce incidence of food borne illnesses by creating multiple barriers. Further understanding of the ecology of *E. coli* O157 in cattle and factors that affect gut persistence and fecal shedding in cattle are important in achieving elimination or significant reduction in pathogen load in cattle presented for slaughter.

Pre and Post Harvest Food Safety Interventions from a Processor's Perspective

Dan Schaefer, Assistant Vice President, Beef Research and Development, Cargill Meats

The 1993 outbreak of *E. coli* O157:H7 was a watershed event for the beef industry that initiated enormous efforts towards reduction, and potential elimination, of this organism from beef products. Today large processors employ significant, post harvest mechanical intervention technologies, such as hide on carcass wash systems, pre-evisceration washes and organic acid sprays, final wash systems, and thermal treatment systems. This is in addition to continuous employee training and monitoring, rigorous Hazard Analysis Critical Control Point (HACCP) systems, and regulatory oversight by the USDA, Food Safety Inspection Service (FSIS). Analogous to milk pasteurization in the early 20th century, the beef industry will require significant technology breakthroughs to eliminate *E. coli* O157:H7. Vaccine technologies for food safety are one of the potential pre-harvest technologies being explored.

Cargill coordinated and partially funded the first large scale commercial application of a food safety vaccine during the summer of 2010. The vaccine was manufactured by Epitopix, LLC of Willmar, Minnesota and is currently licensed and marketed by Pfizer Animal Health. The vaccine was administered, under conditional license, in two doses. The first dose was administered at receiving of the feeder calves into the feedlot and the second dose about 90 days prior to harvest. This test was designed to determine the effects of a whole feedlot food safety vaccination program on antibody titers, fecal shedding, and beef trimmings.

RESULTS:

	Control	Vaccinated	p value
Antibody titer, S:P units	0.075	0.622	p<0.001
Fecal prevalence, %	21.5	13.9	p=0.07
Trim prevalence	Prevalence too low for accurate comparison		

The vaccinated animals showed significantly higher antibody titers at harvest than the control animals (p<0.001). The titer levels of the vaccinated animals correspond to levels in previous small pen trials. Prevalence of *E. coli* O157:H7 in feces collected from feedlot fecal pats, ranged from 7% to 23% across the different collection times. Overall, the vaccine treatment trended lower, but not significantly lower (p=0.07), with a prevalence of 13.9% in the vaccinates vs. 21.5% in the controls. The overall prevalence of *E. coli* O157:H7 in the beef trimmings from controls and vaccinates was too low to make any meaningful.

Opportunities to Reduce the Risk of Shedding of *E. coli* O157 by Cattle: Implications for Beef Safety, the Environment and Public Health

Gary M. Weber, Ph.D.
President, Bioniche Food Safety-USA

Background*:*

E. coli O157:H7 began to be identified as a public health issue in the 1980's. While beef and beef products have been the most common single source of illness, indicating cattle are the primary reservoir for this pathogen, outbreaks have also been associated with many food commodities, including dairy products, beverages and produce (Rangel et al. 2005). In addition, outbreaks have been associated with water contamination (Ali, 2004) and direct contact with livestock at fairs and expositions, particularly cattle (Steinmuller, et al. 2006 and Keen et al. 2006). *E. coli* O157:H7 has been identified in wildlife although it is uncertain if these infections are simply spill over from domestic cattle or sustainable infections. There is a distinct seasonal profile and relationship between shedding of *E. coli* O157:H7 by cattle, beef contamination and human illness associated with various food sources and human contact with livestock.

Seasonal *E. coli* O157:H7 Shedding and Relationship to Human Illness:

It has been well documented that there is a seasonal shedding pattern of *E. coli* O157:H7 in cattle and that there is a corresponding correlation with the occurrence of the pathogen in ground beef and human illnesses resultant from this pathogen. Historically, the peak shedding period for

E. coli O157:H7 in U.S. cattle is June with the corresponding peak of the pathogen in ground beef and human illness in July, (Williams et al. 2010). Other models also indicate a positive correlation between human illness and the carriage of *E. coli* O157:H7 by cattle (Withee, et al. 2009).

From 1991-2002 there have been 183 produce associated outbreaks associated with *E. coli* O157:H7 and 74 percent of these have occurred from July to October (Rangel et al. 2005). The question remains, are these outbreaks linked to the seasonal peak in shedding of *E. coli* O157:H7 by cattle.

Cooley et al. (2007) report that from 1995-2006 there have been 22 *E. coli* O157:H7 contaminated produce outbreaks in the United States and half of these were traced to lettuce and spinach grown in California. Outbreaks between 2002 and 2006 were investigated and possible sources of pre-harvest contamination were identified. A survey of the Salinas valley watersheds indicated *E. coli* O157:H7 was identified at least once from 15 of 22 different watersheds over a 19 month time period.

Steinmuller et al. (2007) reviewed 55 outbreaks of *E. coli* O157:H7 associated with human contact with animals at fairs and exhibitions, and petting zoos. Keen et al. 2007 surveyed *E. coli* O157:H7 prevalence at U.S. fairs. They collected 2,919 fecal specimens at 29 county fairs in 2 states and at 3 state fairs in 2002.

They isolated *E. coli* O157:H7 from livestock at 31 (96.9 percent) of 32 fairs, including 11.4 percent of 1,407 cattle, 1.2 percent of 1,102 swine, 3.6 percent of 364 sheep and goats. These data illustrate the prevalence of *E. coli* O157:H7 in cattle is much higher than in other species.

Is *E. coli* O157:H7 infection in wildlife a spill over infection or sustainable?

Laegreid et al. (1999) report results of sampling range cow operations in Kansas, Missouri, Nebraska and South Dakota. They found 87 percent of herds were found to have at least one

E. coli O157:H7 positive fecal sample with prevalence ranging from 1.7 – 20 percent with an average of 7.4 percent. Serologic evidence suggests 83 percent of calves and 100 percent of cattle herds had been exposed to *E. coli* O157:H7.

During a corresponding time period in Nebraska, Renter et al. (2001) reported 1,608 deer were sampled at harvest during the hunting season and *E. coli* O157:H7 was identified in 0.25 percent of samples. Godfroid, (2002) discussed the relationship of Brucellosis infection from the primary host, cattle, to wildlife as a situation where one must distinguish between a spillover infection from domestic animals and a sustainable infection in a wild species. The question remains, is the presence of *E. coli* O157:H7 in wildlife, or other species of domestic livestock for that matter, a spillover from domestic cattle or is it a sustainable infection?

Williams et al. (2010) state that if cattle are the primary source of *E. coli* O157:H7 and if pre-harvest controls for *E. coli* O157:H7 are effective, there will likely be ancillary benefits such as less contamination

of other food sources such as produce, water and through direct contact with cattle at fairs or exhibitions. This may also result in less infection of other domestic livestock species and wildlife.

How can colonization of cattle and shedding by *E. coli* O157:H7 be reduced?

Rosenshine et al. (1996) and others have identified the mechanisms whereby *E. coli* O157:H7 can trigger epithelial cells to form bacterial receptors that mediate actin pseudopod formation. These formations are central to the colonization of the intestinal mucosa by this pathogen. Potter et al. (2004) and Peterson et al. (2007) have demonstrated that a vaccine containing the antigens associated with actin pseudpod formation (secretory proteins: EspA, EspB, Tir, Intimin) will produce a dose related IgG response.

In controlled challenge studies required to license an *E. coli* O157 vaccine in Canada, 3 doses of a vaccine containing these antigens reduced the magnitude of shedding in vaccinated cattle, as compared to controls, by 2.28 logs (99 percent) and the number of days *E. coli* O157 was shed after oral challenge by 63.9 percent (Rogan et al. 2009).

Numerous field trials in the United States with a vaccine containing these secretory proteins have demonstrated a dose related reduction in colonization by as much as 98 percent (Peterson et al. 2007), probability of cattle shedding *E. coli* O157 in feces by as much as 65 – 73 percent (Moxley, et al. 2009 and Peterson et al. 2007) and hide contamination by as much as 54 percent (Smith et al. 2009). It is important to note that in these field studies, vaccinated cattle were routinely exposed to a variable oral challenge from *E. coli* O157 shed by non-vaccinated cattle.

It is theorized that whole herd vaccination would further reduce the natural oral challenge and correspondingly increase the observed efficacy of vaccination.

Would a reduction in shedding of *E. coli* O157:H7 by cattle reduce human illness?

At the Beef Industry Food Safety Council (BIFSCo) Beef Industry Food Safety Summit held in 2007, Dr. David Smith reported that in modeling the potential impact of a vaccine with a 65 percent efficacy (reduction in the probability of shedding, Moxley, et al. 2009), by feedlot cattle the net impact would be to convert the summer shedding profile of cattle to more of a winter prevalence profile.

The data analysis provided by Williams et al. (2010) supports the theory that a reduction in the peak shedding period for *E. coli* O157:H7 by cattle, as observed from April through September in the U.S., to that observed from October through March, would correspondingly reduce the prevalence of *E. coli* O157:H7 in beef and reduce associated human illness. Evidence suggests that post harvest (in-plant) interventions are currently capable of controlling the risk posed by

E. coli O157:H7 contamination of beef from October through March. However, these intervention systems appear to be overloaded as a result of the seasonal increase in shedding from April through September. Research indicates vaccination of cattle would reduce this seasonal burden. In addition, if there was wide spread adoption of vaccination, it is reasonable to expect the reduction in shedding of *E. coli* O157 by cattle would correspond to a reduction in human illness associated with produce, water and contact with livestock, particularly cattle, as well as other species.

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Overview of Meat Regulations Regarding *E. coli* 0157:H7

William James, DVM, MPH, Chief Public Health Veterinarian, USDA-FSIS

E. coli serotypes that have resulted in foodborne outbreaks are those that produce the shiga toxin (Stx). Stx producing *E. coli* (STECs) have a number of characteristics that make them dangerous. They can be very hardy, able to live on various surfaces for several weeks, and have a very low infectious dose. The most notorious Stx producing *E. coli* is *E. coli* O157:H7 and is responsible for the majority of human illnesses attributed to *E. coli.* Foods identified as sources of contamination include ground beef, sausages, unpasteurized milk & cheese, unpasteurized apple juice, orange juice, alfalfa & radish sprouts, lettuce, and spinach.

Surveillance for O157 STECs has been improving over time. The estimated incidence of STEC O157 infections observed in 2009 is similar to that observed in 2004, having increased and then decreased in the interval (~1case/100,000 persons).

The Food Safety and Inspection Service (FSIS) considers raw ground beef products contaminated with *E. coli* O157:H7 to be adulterated and not eligible to bear the mark of inspection.

As of October 24, 2010 a total of 9,565 ground beef product samples have been collected in federally inspected establishments and tested for *E. coli* O157:H7. The percentage of positive product samples was 0.27%. As of October 24, 2010 a total of 2,246 ground beef component samples collected in federally inspected establishments and tested for *E. coli* O157:H7. The percentage of positive product samples was 0.31%.

On October 5, 2009 FSIS was petitioned to issue a rule declaring all STECs, including non-0157 serotypes, to be adulterants within the meaning of the Federal Meat Inspection Act. FSIS scientists are examining all available data to develop a set of recommendations to the Administrator on how FSIS should proceed with respect to non-0157 STECs. When FSIS has developed a plan for how it intends to address this issue, it will make the plan available to the public for comment.