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What are we doing about *Escherichia coli* O157:H7 in cattle?[1,2]

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ABSTRACT: Many human foodborne illnesses can be caused by consumption of foodstuffs (including meat products) contaminated with pathogenic bacteria from animal intestinal contents or hides. Steps that have been taken in the slaughter plant to decrease the spread of foodborne pathogenic bacteria (e.g., hazard analysis and critical control point methods) have been very effective; however, meat products are still the source of foodborne bacterial human illnesses. Increasing numbers of human *Escherichia coli* O157:H7 illnesses have also been related to contact with animals or to water supplies contaminated by run-off from cattle farms. Thus, strategies that specifically target foodborne pathogenic bacteria in the animal at the farm or feedlot level have great potential to improve food safety and decrease human illnesses. In this review, we describe a broad range of live-animal intervention strategies, both probiotic and antipathogen. Additionally, we examine some of the effects of diet and management strategies on foodborne pathogenic bacterial populations. The use of antibiotics in food animals to decrease foodborne pathogens also will be briefly examined. Overall, the concurrent use of several of these preslaughter intervention strategies could synergistically decrease human illnesses by providing for additional barriers in a multiple-hurdle approach to improving food safety.

Key Words: Foodborne Diseases, Intervention, Pathogens

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Introduction

The food supply in the United States is indeed the safest in the history of the world, yet each year more than 76 million citizens become ill from consuming foods contaminated with pathogenic bacteria (Mead et al., 1999). Some of these outbreaks have been linked to contact with cattle and/or their waste or consumption of contaminated meat products. Many pathogenic bacteria are part of the normal gastrointestinal microbial population of cattle, including *Escherichia coli* O157:H7 and other enterohaemorrhagic *E. coli* (**EHEC**).

For many years, the cattle industry and researchers have focused on improving the safety of meat products after slaughter. Postslaughter antimicrobial treatments and HACCP policies in slaughter plants have been shown to significantly reduce carcass contamination (Elder et al., 2000). Yet, in spite of the tremendous strides made in the processing plants, illnesses caused by contaminated meat products still occur. Therefore, greater emphasis has recently been placed on the development of intervention strategies that target the pathogenic microbial population of the live animal before slaughter.

Fecal Shedding in Cattle as a Route to Human Populations

Fecal shedding of *E. coli* O157:H7 in cattle is directly correlated with levels of carcass contamination (Elder et al., 2000), emphasizing that the live animal is a critical link in the production chain. Additionally, increasing numbers of human illness outbreaks have been correlated with animal contact at fairs, and EHEC have been isolated from ruminants at a large number of agricultural fairs across the United States (Keen et al., 2003). Reducing the pathogen burden entering the abattoir, at agricultural fairs, and in cattle farm run-off could produce “the most significant reduction in human exposures to the organism and therefore reduction in

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[2]Proprietary or brand names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by the USDA implies no approval of the product, and/or exclusion of others that may be suitable.

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related illnesses and deaths" (Hynes and Wachsmuth, 2000).

Unfortunately, diagnosing cattle on the farm or the feedlot as being "infected" by pathogenic bacteria is not an easy task because these pathogens often have little or no effect on the health or production efficiency of the animal. In the case of *E. coli* O157:H7, it has been found that cattle are insensitive to the deleterious effects of the toxins produced by *E. coli* O157:H7 and other EHEC (Pruimboom-Brees et al., 2000). Detection of *E. coli* O157:H7 is also complicated by the fact that fecal shedding can be very sporadic, with an animal testing positive for EHEC one day, but not again for several days or even weeks. Additionally, diagnostic tests for EHEC in cattle feces can be quite expensive and time consuming. Therefore, strategies to decrease pathogen levels in animals cannot be focused on a small, diagnosed subpopulation. Rather, they must be applicable to large groups of animals at different phases of production or immediately before slaughter.

This review examines several different strategies to reduce foodborne pathogens in cattle. Intervention methodologies can be loosely clustered into probacterial and antipathogen strategies, as well as dietary and management strategies. Some of these strategies could potentially be used in combination to achieve a synergistic reduction in foodborne pathogenic bacteria.

Potential Probacterial Intervention Strategies

Probiotics are defined as commensal (harmless or beneficial) bacteria used to reduce pathogenic bacteria in the gut (Fuller, 1989). Commensal organisms in the gut can be competitive or antagonistic to foodborne pathogenic bacteria. In general, probacterial strategies can be categorized into two groups: 1) the introduction of a "normal" (nonpathogen containing) intestinal microbial population (probiotics) or 2) providing a limiting substrate (a prebiotic) that is not digestible by the host animal but which may allow an already existing microbial population to expand its niche in the gastrointestinal population. In this review, we will only discuss the use of probiotics in cattle.

Probiotics in general have not always been widely commercially implemented due to the inconsistent nature of "real-world" results. Often, some of the conflicting data were due to the antagonistic effect of the use of some other management techniques, such as the use of antibiotics (Steer et al., 2000). Because of increased concerns about the issue of antimicrobial resistance, it is expected that the prophylactic use of antibiotics as growth promotants in cattle will decrease in the future, causing strategies employing a "probacterial" slant to become increasingly utilized.

Competitive Exclusion

Competitive exclusion (**CE**) as a pathogen-reduction technology is simply the addition of an exogenous bacterial population (nonpathogenic) to the intestinal tract of the animal in order to reduce colonization or decrease existing populations of pathogenic bacteria in the gastrointestinal tract (Fuller, 1989; Nurmi et al., 1992; Steer et al., 2000). The CE culture may be composed of a single or multiple strains of a single bacterium, or even several different species of bacteria. Endogenous gastrointestinal bacteria compete with one another for available nutrients (Hungate, 1966). The best-adapted species flourishes in each and every niche of the intestinal tract. It is thought that the addition of a mixed CE culture limits the populations of pathogenic bacteria through three general mechanisms: 1) competition for limiting nutrients, 2) direct competition for binding sites along the gut epithelium, or 3) production of toxic compounds (e.g., VFA, bacteriocins, or antibiotics) (Nurmi et al., 1992; Crittenden, 1999; Steer et al., 2000).

Applications of Competitive Exclusion and Probiotics in Cattle

Competitive exclusion has been widely used in poultry to prevent *Salmonella* colonization of broilers (Nurmi and Rantala, 1973; Nurmi et al., 1992). Recent studies demonstrating the effectiveness of CE in reducing *Salmonella* colonization of chicks has led to the commercial development in the United States of a defined, mixed-culture CE product comprised of several species of bacteria (Preempt, MS BioScience, Dundee, IL) (Nisbet et al., 1993a,b; 1996). Research has also shown that CE can be effective in preventing *Salmonella* and enterotoxigenic *E. coli* colonization of swine (Anderson et al., 1999; Fedorka-Cray et al., 1999; Genovese et al., 2000). Ongoing field trials with a porcine-derived commercial CE product for swine have been highly encouraging (Harvey et al., 2003).

Historically, CE was not considered a viable technique for cattle because of the vast microbial reservoir of the rumen and the long production cycle of cattle. However, recent research has demonstrated that CE and other probiotics could be effectively used to reduce *E. coli* O157:H7 and other bacteria in cattle (Zhao et al., 1998; Tkalcic et al., 2003; Zhao et al., 2003). Researchers isolated a population of several *E. coli* strains from cattle that did not contain *E. coli* O157:H7 (Zhao et al., 1998). During in vitro studies, they found that this generic *E. coli* culture produced colicins (proteins that specifically target *E. coli*), and in vivo studies found that this culture could displace established *E. coli* O157:H7 populations from adult cattle (Zhao et al., 1998) and could reduce fecal shedding of EHEC in neonatal calves (Zhao et al., 2003) and in weaned calves (Tkalcic et al., 2003). This cattle CE culture is currently being developed as a commercial product, and field trials are expected to be underway within a year.

Other researchers have found that the addition of a probiotic *Lactobacillus acidophilus* culture to the diet of finishing feedlot cattle decreased *E. coli* O157:H7 shedding by more than 50% (Brashears et al., 2003a). Additional feedlot studies have shown that this culture reduced fecal shedding of *E. coli* O157:H7 from 21 to 13%, but this decrease was not statistically significant (Moxley et al., 2003). Although this *Lactobacillus* culture is not a true CE culture per se (rather, it is considered to be a direct-fed microbial **[DFM**], it is highly encouraging that probiotic administration can decrease shedding of *E. coli* O157:H7 in the live animal (Brashears et al., 2003a,b). This DFM product is currently available on the market, and is being used in the cattle industry.

Potential Antipathogen Intervention Strategies

In an effort to rid pathogenic bacteria from cattle, it is logical to envision the use of strategies that specifically target and kill pathogenic bacteria. There are several potential antipathogen strategies that have been investigated in recent years, and these are: 1) use of traditional antibiotics, 2) use of antimicrobial proteins produced by bacteria, 3) use of bacteriophage, 4) use of compounds that specifically target the physiology of pathogenic bacteria, and 5) vaccination strategies.

Traditional Antibiotics

Antibiotics are commonly included in animal rations, and are widely used to treat illnesses in cattle. However, the use of antibiotics as growth promotants has become highly controversial in recent years and is likely to become more so in the near future following recent regulatory actions by the European Union. Bacteria have many complex mechanisms to resist antibiotics, and the widespread use of antibiotics in both human medicine and animal agriculture has led to the widespread dissemination of antibiotic resistance genes. Because of concern over the spread of antibiotic resistance, it is likely that the prophylactic use of antibiotics as growth promotants in food animals will become even more highly regulated or even completely prohibited in the United States. Additional pressures will likely also be brought to bear on the cattle feeding industry, as evidenced by the recent decision by McDonald's to not purchase meat from producers who use growth-promoting antibiotics (McDonald's, 2003). Further information on this controversial topic can be found in the related symposia by Salyers and Bischoff.

However, some antibiotics have been shown to directly affect intestinal populations of pathogenic bacteria. When cattle were treated with neomycin sulfate, shedding and fecal populations of *E. coli* O157:H7 were significantly decreased (Elder et al., 2002). Further follow-up studies by K. Belk at Colorado State University have confirmed that neomycin treatment can significantly decrease fecal shedding of *E. coli* O157:H7 (Ransom et al., 2003). Neomycin has only a 24-h withdrawal period before slaughter and is not of important use in human medicine; however, it is closely related to other antibiotics in the aminoglycoside family (e.g., streptomycin, kanamycin, and gentamycin) that are used to treat some human infections (e.g., drug-resistant tuberculosis) (Bhasi, 2001). Thus, resistance and cross-resistance issues must still be considered before widespread implementation of this practice. It seems that neomycin could be immediately used in the cattle industry to decrease *E. coli* O157:H7 populations in finishing cattle, but only until other, more desirable and less controversial, intervention strategies become available. At the time of writing, this off-label use of neomycin is not approved by the FDA; however, discussions are underway to change the labeling of neomycin to allow its use to reduce *E. coli* O157:H7.

Effect of Ionophores on Pathogen Populations in Cattle

Ionophores are growth-promoting antimicrobials that are widely used in cattle production to increase production efficiency (Russell and Strobel, 1989). Ionophores are not related to, and do not share a common mode of action with, antibiotics used in human medicine and are therefore unlikely to contribute to an increase in antibiotic resistance (Russell and Houlihan, 2003). Studies have indicated that the development of resistance to ionophores is a physiologic selection rather than a genetically mediated transformation (Callaway et al., 1999; Callaway and Russell, 2000; Russell and Houlihan, 2003). Gram-positive bacteria are primarily inhibited by ionophores, yet many foodborne pathogens of human interest (e.g., *Salmonella* and *E. coli*) are gram-negative and are ionophore-insensitive (Edrington et al., 2003c). Concerns have been raised about ionophores providing a competitive advantage to the gram-negative species (including pathogens). In vitro and in vivo studies have demonstrated that this is not the case with respect to *Salmonella* and *E. coli* in ruminant animals (Edrington et al., 2003b,c).

Bacteriophages as a Method to Decrease Foodborne Pathogens

Bacteriophages are viruses that specifically kill bacteria and are common members of the intestinal microbial flora of food animals. Phage have been repeatedly isolated from the bovine and ovine rumen and intestine (Klieve and Bauchop, 1988; Klieve et al., 1991; Klieve and Swain, 1993). Recently, 46% of sheep transported from open rangeland were found to be naturally infected with bacteriophages active against *E. coli* O157:H7 (Callaway et al., 2003b).

Bacteriophages are highly specific and can be active against a single strain of bacteria (Barrow and Soothill, 1997). Therefore administration of a bacteriophage to cattle (and other food animals) has been suggested to specifically eliminate pathogens from a mixed microbial population (Merril et al., 1996; Summers, 2001). Phages have been used successfully in several in vivo research studies examining the effect of phage use on diseases that impact production efficiency or health in swine, sheep, and poultry (Smith and Huggins, 1983, 1982; Huff et al., 2002). Enteropathogenic *E. coli* (**EPEC**) cause diarrhea in cattle and are similar in some physiological and ecological respects to *E. coli* O157:H7. Bacteriophage treatment decreased EPEC-catalyzed diarrhea and splenic EPEC colonization in calves (Smith and Huggins, 1987, 1983), indicating that bacteriophages could be useful in the effort to reduce foodborne pathogenic bacteria entering the food chain.

Some researchers have examined the use of bacteriophage to specifically decrease *E. coli* O157:H7 in cattle (Kudva et al., 1999; Bach et al., 2002). Several O157-specific phages were isolated, but were only active under highly aerated conditions (Kudva et al., 1999). These highly aerated conditions could be easily achieved during the treatment of foods (e.g., sprouts), but not within the gastrointestinal tract of food animals. A similar pattern of in vitro success, followed by lower effectiveness in vivo, was observed in other phage experiments (Bach et al., 2002). In further studies, bacteriophages specific against *E. coli* O157:H7 were isolated from sheep and were added to sheep experimentally infected with *E. coli* O157:H7 (Callaway et al., 2003b). Bacteriophage treatment decreased concentrations of *E. coli* O157:H7 throughout the gastrointestinal tract; although these differences were not statistically significant, they were encouraging as a "proof of concept" for the use of bacteriophages to control foodborne pathogens in the ruminant gastrointestinal tract (Callaway et al., 2003b). The effectiveness of phage treatment in "real-world" conditions has been variable; therefore, more basic work needs to be completed before bacteriophages can be considered a viable method to control foodborne pathogenic bacteria in cattle.

Inhibition of Specific Pathogens via Metabolic Pathways

Salmonella and *E. coli* can respire under anaerobic conditions by reducing nitrate to nitrite via a dissimilatory nitrate reductase (Stewart, 1988). The intracellular bacterial enzyme nitrate reductase does not differentiate between nitrate and its analog, chlorate which is reduced to chlorite in the cytoplasm; chlorite accumulation kills bacteria (Stewart, 1988). Chlorate addition to swine and sheep diets reduced experimentally inoculated *Salmonella* and *E. coli* O157:H7 populations in feces and intestinal contents (Anderson et al., 2001a,b; Edrington et al., 2003a). Other studies indicated that chlorate administered in drinking water significantly decreased *E. coli* O157:H7 ruminal, cecal, and fecal populations in both cattle and sheep (Callaway et al., 2002, 2003a).

Results have indicated that chlorate treatment does not affect the ruminal or the cecal/colonic fermentation in ruminant or monogastric animals (Anderson et al., 2000b, 2002; Callaway et al., 2002). Because of the dramatic impact chlorate has on foodborne pathogenic bacterial populations in the gut of food animals, it has been suggested that chlorate could be supplemented in the last meal (approximately 24 h before slaughter) before shipment to the slaughterhouse (Anderson et al., 2000a). The use of chlorate in food animals is presently under review by the U. S. FDA, but has not been approved for use in food animals.

Immunization to Prevent Pathogen Shedding and Colonization

Methods to exploit the animal's own immune system to decrease pathogen populations in the gastrointestinal tract have also been studied extensively. Specific immunization against pathogenic bacteria has shown great promise in reducing the levels of disease causing pathogens in food animals, but has only recently been used to attempt to decrease intestinal populations of foodborne pathogenic bacteria in cattle. Vaccines against *Salmonella* strains responsible for disease have been previously used in swine and dairy cattle (House et al., 2001). However, because many foodborne pathogenic bacteria do not cause illness in the host animal, development of vaccines against these pathogens has been a difficult process. Recently, however, a vaccine has been developed for use in feedlot cattle that significantly decreases fecal *E. coli* O157:H7 shedding (Finlay, 2003). Preliminary experimental results indicated that this vaccine decreased *E. coli* O157:H7 shedding in feedlot cattle from 23% to less than 9% (Moxley et al., 2003). This vaccine is currently undergoing field trials before further development as a commercial product for feedlot cattle.

As discussed previously, if two complimentary intervention strategies could be combined, a synergistic decrease in pathogens could theoretically be obtained. Unfortunately, a well-controlled study examining concurrent use of the Finlay vaccine and the Brashears et al. (2003b) *Lactobacillus* DFM/CE culture (discussed above) did not reveal any synergistic benefits to the use of these two intervention strategies (Moxley et al., 2003). Therefore, although some technical issues remain to be resolved, the use of vaccination to decrease foodborne pathogens seems to hold significant theoretical promise, and could be used in conjunction with other more directly compatible pathogen-reduction technologies.

Diet and Management Effects

Good animal management is crucial to the production of healthy, efficiently grown cattle. Yet no management strategies have been demonstrated that impact shedding or carriage of foodborne pathogens in cattle. However, decreasing the opportunities for pathogens to multiply in feed and water may reduce horizontal and vertical transmission of pathogens between herd and pen mates (Hancock et al., 1998).

Dietary Changes to Decrease E. coli *O157:H7 Populations in Cattle*

Cattle in the United States are often fed high-grain diets to maximize growth efficiency (Huntington, 1997). Some dietary starches bypass ruminal fermentation and pass through to the cecum and colon, where they undergo a secondary microbial fermentation (Huntington, 1997). The type of grain used in a finishing ration can significantly impact fecal shedding of *E. coli* O157:H7. For example, barley feeding has been linked to increased shedding of *E. coli* O157:H7 (Dargatz et al., 1997).

When cattle were abruptly switched from a finishing ration to a 100% hay diet, fecal *E. coli* populations and the population of acid-shock resistant *E. coli* declined significantly within 5 d (Diez-Gonzalez et al., 1998). Based on these results, it was suggested that feedlot cattle be switched from high-grain diets to hay immediately before slaughter to reduce *E. coli* overburden in the abattoir (Diez-Gonzalez et al., 1998). In a similar study, Keen et al. (1999) divided cattle naturally infected with O157:H7 into two groups: one maintained on a feedlot ration and the other abruptly switched to hay. Of the grain-fed cattle, 52% remained *E. coli* O157:H7-positive compared with 18% of the cattle abruptly switched to hay (Keen et al., 1999). Based on these and other results, it has been stated that "the most effective way of manipulating gastrointestinal counts of *E. coli* was to feed hay" (Gregory et al., 2000).

Unfortunately, other results have indicated that longer-term forage feeding had no effect or even increased *E. coli* O157:H7 shedding (Hovde et al., 1999; Buchko et al., 2000a,b). However, based on the available literature, it seems that an abrupt shift to forage feeding decreases *E. coli* populations, but the magnitude of this effect is not always consistent, and the controversy over this topic continues (Callaway et al., 2003c). The significance of this "forage effect" must be carefully weighed against the impact on carcass quality and economic and other infrastructure factors.

Water Systems and Runoff as a Reservoir of E. coli *O157:H7*

Cattle (and humans) can be infected with pathogenic bacteria via a water-borne route (Jackson et al., 1998; Shere et al., 2002). In some very well-designed studies, researchers have demonstrated that cattle water troughs can be reservoirs for dissemination of *E. coli* O157:H7 (LeJeune et al., 2001). Although the significance of this route of horizontal transmission has not been decisively proven in cattle, interventions at the water trough level offer significant potential to decrease *E. coli* O157:H7 contamination and cross-contamination of animals (LeJeune et al., 2001). Suggested potential strategies to reduce *E. coli* O157:H7 survival in the water supply include chlorination, ozonation, frequent cleaning, and screens that decrease organic solids in the troughs.

Waterborne human *E. coli* O157:H7 outbreaks have become more common in recent years, with several human outbreaks linked to water contamination by cattle feces (Anonymous, 2000). Additionally, water run-off from farms contaminated with pathogenic bacteria can be used to irrigate feed crops where it can later be consumed by animals or human consumers (Maule, 2000; Sanchez et al., 2002). Further research into reducing pathogen survival and multiplication in the water supply and in farm runoff can potentially increase food safety by reducing the risk of foodborne pathogen horizontal transmission via drinking water.

Implications

The American beef cattle industry goes to remarkable lengths to provide a safe product; however, foodborne illnesses related to meat products or contact with cattle still occur. Until recently, much research focused on postslaughter intervention strategies, but this has now changed following the development of several potential preharvest intervention strategies. The use of vaccination, probiotics, competitive exclusion, antibiotics, antimicrobials, bacteriophage, sodium chlorate, changing dietary practices, and good animal management can potentially decrease the incidence of foodborne pathogenic bacteria that enter the abattoir. Further research into interventions that focus on this preslaughter "critical control point" is vital to improving overall food safety and resultant human health. Although some of these intervention strategies are currently available, more will soon become available to assist producers as they strive to produce a safe, wholesome, and high-quality product.

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