Preharvest control of *Escherichia coli* O157 in cattle\(^1\)

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**ABSTRACT:** Bovine manure is an important source of *Escherichia coli* O157 contamination of the environment and foods; therefore, effective interventions targeted at reducing the prevalence and magnitude of fecal *E. coli* O157 excretion by live cattle (preharvest) are desirable. Preharvest intervention methods can be grouped into 3 categories: 1) exposure reduction strategies, 2) exclusion strategies, and 3) direct antipathogen strategies. Exposure reduction involves environmental management targeted at reducing bovine exposure to *E. coli* O157 through biosecurity and environmental niche management such as feed and drinking water hygiene, reduced exposure to insects or wildlife, and improved cleanliness of the bedding or pen floor. In the category of exclusion, we group vaccination and dietary modifications such as selection of specific feed components; feeding of prebiotics, probiotics, or both; and supplementation with competitive exclusion cultures to limit proliferation of *E. coli* O157 in or on exposed animals. Direct antipathogen strategies include treatment with sodium chlorate, antibiotics, bacteriophages, in addition to washing of animals before slaughter. Presently, only 1 preharvest control for *E. coli* O157 in cattle has been effective and has gained widespread adoption—the feeding probiotic *Lactobacillus acidophilus*. More research into the effectiveness of parallel and simultaneous application of 1 or more preharvest control strategies, as well as the identification of new preharvest control methods, may provide practical means to substantially reduce the incidence of human *E. coli* O157-related illness by intervening at the farm level.

**Key words:** cattle, *Escherichia coli* O157, food safety, preharvest


**INTRODUCTION**

Foods of bovine origin have frequently been linked to cases of human illnesses due to *Escherichia coli* O157:H7 infections. The cattle industry and researchers have invested much time and resources on improving the safety of meats at the time of harvest and processing. Implementation of hazard analysis and critical control point policies and enhanced postslaughter sanitation methods are temporally correlated with a reduction in the frequency that ground beef is contaminated with this foodborne pathogen (CDC, 2005; USDA, 2005). Despite adoption of in-plant intervention strategies focused specifically to reduce this pathogen and other foodborne pathogens in the finished meat product, illnesses are continuously being reported. Furthermore, in-plant intervention strategies do not affect the frequency of human exposure to this pathogen from direct contact with cattle nor via indirect exposure to the organism from manure-contaminated foods such as fruits and vegetables, water, or the environment.

Although testing and segregation of *E. coli* O157:H7-positive cattle near or immediately before slaughter until the animals test negative has been considered as a method to limit the number of cattle contaminated with *E. coli* O157:H7 entering the food chain, this approach is problematic for several reasons, including 1) the sporadic pattern of fecal excretion of *E. coli* O157:H7 is such that an animal might test negative one time but be reexposed before the next test period or slaughter; 2) the hide may be contaminated in the absence of fecal excretion, and 3) the expense and logistics of handling large numbers of test-positive animals is prohibitive. Thus, considering the diverse sources and routes through which humans may contact contaminated bovine manure, intervention strategies that target the pathogen in live animals on the farm before slaughter, termed preharvest intervention or control, may have the largest impact on improving beef safety.

**PREHARVEST INTERVENTION CONCEPTS**

The concept of preharvest intervention to control microbial hazards in food-producing animals is not new.
One of the most serendipitous but successful examples of preharvest intervention was the US ban on the feeding of uncooked garbage to swine to prevent hog cholera and vesicular exanthema. These changes, instituted in the 1950s, resulted in decreasing prevalence of *Trichinella* in swine and, because undercooked contaminated pork was the primary vehicle of human exposure, a concurrent precipitous drop in human trichinosis cases in the United States (Roy et al., 2003; Pyburn et al., 2005). Analytical and theoretical models of preharvest control of *E. coli* O157:H7 have likewise predicted significant impacts of on-farm intervention strategies on human cases of this foodborne bacterium (Jordan et al., 1999a,b). More recently, empirical data collected by Elder et al. (2000) confirm the importance of lowering the preharvest prevalence of *E. coli* O157 on the carcass contamination rate. In recent years, the frequency of *E. coli* O157 contamination in ground beef has declined, and this decrease is concurrent with a decrease in the incidence of infections caused by *E. coli* O157; however, it has not been possible to demonstrate that these 2 parameters are causally associated with one another.

Unfortunately, several epidemiological and ecological characteristics of *E. coli* O157:H7 represent constraints to the effectiveness of some strategies used for preharvest control of *E. coli* O157:H7. The widespread distribution of *E. coli* O157:H7, its persistence in environmental sources, its apparent ability to reinfect cattle, as well as its wide host range (including wildlife), make complete eradication of *E. coli* O157:H7 an unrealistic goal (Hancock et al., 2001). An achievable objective for preharvest intervention for *E. coli* O157:H7 in cattle is to reduce the magnitude or the prevalence of fecal excretion, or both. These goals may be reached by limiting farm-to-farm dissemination of the organism, cattle-to-cattle dissemination within a farm, or proliferation within an individual animal. These preharvest intervention methods can be grouped into 3 general categories: 1) exposure reduction strategies; 2) exclusion strategies, and 3) direct antipathogen strategies. Some of these preharvest intervention strategies are available for application, some will be available in the near future, and other strategies still require more research into their effectiveness at controlling *E. coli* O157:H7 before they can be available to livestock producers.

**EXPOSURE REDUCTION STRATEGIES**

*Escherichia coli* O157:H7 has been isolated from a large number of animals and objects in the farm environment including manure, soils, feeds, water sources, bedding, dust, flies, wildlife, and the hides of cattle. Although often considered to be ubiquitous in the farm environment, some farms tend to consistently test positive for *E. coli* O157 more frequently than others (Hancock et al., 1997). It is logical that reducing the frequency of exposure of cattle to these contaminated sources will reduce *E. coli* O157:H7 prevalence in live animals. However, at present, it is not known how much each of these potential sources of exposure contributes to the overall herd prevalence of this organism.

**Water Quality**

Several researchers have demonstrated a positive association between drinking water contamination with *E. coli* O157:H7 and the presence of this organism in cattle feces (Faith et al., 1996; LeJeune et al., 2004; Davis et al., 2005). When water sources are positive for *E. coli* O157, inevitably cattle are also positive for this organism, and vice versa. Clearly, water sources for cattle are frequently contaminated with relatively high numbers of generic *E. coli*, and water troughs can be reservoirs and vehicles for dissemination of *E. coli* O157:H7 (LeJeune et al., 2001a,b). Whether the presence of *E. coli* O157 in these troughs is simply a result of bovine fecal contamination or a principle source of exposure to this foodborne pathogen remains undetermined. Attempts to enhance livestock drinking water quality by various treatment methods including chlorination, electrolyzed water, and ozonation have yielded marginal impacts on water quality variables (LeJeune et al., 2004), poor palatability (Zhao et al., 2006), or negligible effectiveness on *E. coli* O157:H7 prevalence in cattle when tested in the field (LeJeune et al., 2004; Besser et al., 2005; Zhao et al., 2006). Furthermore, coliform or *E. coli* contamination levels in water are not predictive of *E. coli* O157:H7 presence (LeJeune et al., 2004; Sanderson et al., 2005). Although eliminating *E. coli* O157:H7 from cattle drinking water may be a meritorious goal, until such time that practical, economical, and effective measures that reduce livestock drinking water contamination have been demonstrated to significantly affect the epidemiology of *E. coli* O157 or other pathogens in cattle are demonstrated, investment in extensive water trough management programs may not be warranted from a food safety standpoint.

**Feed Hygiene**

Similar to water contamination, the hygiene of livestock feeds are thought to play key role in the exposure of cattle to *E. coli* O157:H7 (Crump et al., 2002). Feed previously exposed to cattle, as well as feed components intended for cattle feeds, have been reported to be contaminated with *E. coli* O157:H7 with prevalence values approaching 1 and 15%, respectively (Davis et al., 2003; Dodd et al., 2003). To date, however, there have been no published reports describing a correlation among the magnitude or frequency of feed contamination with coliforms, generic *E. coli*, or *E. coli* O157:H7 and bovine prevalence of the organism. Two recent studies failed to find any correlation between the magnitude of coliform contamination of feeds and *E. coli* O157:H7 prevalence in cattle (Dodd et al., 2003; LeJeune et al., 2006). Nevertheless, in several European and Scandinavian countries strict animal feed hygiene controls are in place,
and the incidence of *Salmonella* (also thought to be frequently feedborne) is low in cattle and swine (Boqvist et al., 2003). It remains to be seen if increased feed hygiene results in measurable decreases in *E. coli* O157 and other foodborne pathogen in cattle.

**Environmental Exposure**

Given the natural grooming behavior of cattle and the potential for bacteria to become dispersed on dust particles it would seem logical that the hygienic conditions of the livestock’s immediate environment, such as the floor of feedlot cattle pens or bedding provided for dairy cattle, would have significant impacts on bovine exposure to *E. coli* O157:H7. In fact, Smith et al. (2001) reported that the prevalence of *E. coli* O157 in cattle was greatest in pens that were muddy or wet. Dairy cattle housed in barns with flush-type manure removal systems were more likely to have *E. coli* O157:H7 than animals housed in barns where manure was removed by scraping (Garber et al., 1999).

Although dusty feedlot conditions are not desirable for many environmental, animal health, and human occupational health concerns, decreasing dust formation through sprinkling had no significant impact on fecal shedding of *E. coli* O157:H7 in feedlot cattle (Loneragan and Brashears, 2005; Morrow et al., 2005). Other studies have demonstrated that gram-negative bacteria are infrequently recovered from dust at feedlots, possibly because of their rapid inactivation by UV irradiation (Wilson et al., 2002). In contrast, animals housed indoors may be exposed to gram-negative bacteria on dust particles (Zucker et al., 2000). A greater prevalence of *E. coli* O157:H7 was reported (2005) in dairy cows housed on sawdust bedding compared with herds bedded with sand (P = 0.05). Clearly, given the diverse management difference between beef and dairy cattle, preharvest control measures in these 2 production systems will probably differ significantly.

**Animal Density**

Several studies have identified grouping of calves before weaning as a risk factor for increased fecal shedding of *E. coli* O157:H7 excretion by calves. Calves typically excrete *E. coli* O157 more frequently and in greater numbers than adult animals (Cray and Moon, 1995). Thus, calves may serve a reservoir of *E. coli* O157:H7 from which the organisms are maintained and periodically disseminated into adult animals housed nearby. Housing calves separately, away from other stock, may provide a mechanism to prevent O157 on farms; however, this hypothesis has not been validated. Housing preweaner calves in a physically separate location from older animals may be possible for dairy calves but not for cow-calf pairs on pasture. Off-site heifer raising is another option, but biosecurity risks associated with bringing bred heifers back onto the farm from outside sources must carefully be considered (Hegde et al., 2005).

Most studies that have explored the relationship between herd size and *E. coli* O157:H7 prevalence in dairy herds have not yielded significant correlations between these 2 factors (Dodson and LeJeune, 2005). However, Vidovic and Korber (2006) recently reported a significantly greater *E. coli* O157:H7 prevalence in feedlot cattle that were housed at high density compared with cattle in pens housed with more area per cow.

**Wildlife Exclusion**

*Escherichia coli* O157:H7 has been isolated from a large number of nonbovine sources on farms. This includes other domestic animals (i.e., dogs, other ruminants, horses, and pigs; Hancock et al., 1998). In addition, others have documented *E. coli* O157 in wildlife including raccoons, opossums (Renter et al., 2004), rats (Cizek et al., 1999), wild birds (Nielsen et al., 2004), and deer (Rice et al., 1995). Although cattle are considered the primary reservoir of this organism, it is possible that novel strains of *E. coli* O157:H7 are introduced into cattle populations through feed or water contaminated with the feces from these animals (Daniels et al., 2003; Wetzel and LeJeune, 2006). Once introduced, passage through even a single cow could effectively amplify the organism to levels that could result in a large number of cattle becoming colonized with *E. coli* O157:H7. Thus, exclusion of wildlife from livestock feeds and water would appear to be a beneficial practice. Similar to the situation with feed and water hygiene, the effects of wildlife exclusion, to the extent currently possible, on *E. coli* O157:H7 prevalence in cattle have yet to be documented.

**EXCLUSION STRATEGIES**

There are several methods by which researchers have attempted to modify the microenvironment of the gastrointestinal tract of cattle to prevent the establishment of, or cause the displacement of, particular bacteria. The ultimate goal of these strategies is often based on the concept that beneficial bacteria will fill the same ecological niche of the foodborne pathogens in the gastrointestinal tract, produce a substance or modify the microenvironment of the intestinal tract in such a way that it is inhibitory or deleterious to the targeted pathogens (Fuller, 1989). The most common of these methods includes dietary manipulation based on the use of specific feed components, probiotics, prebiotics, and competitive exclusion agents.

**Feed Component Management**

Feed management has been suggested to influence environmental conditions within the gastrointestinal tract that modify the competitiveness or survival of *E. coli* O157:H7. Early epidemiological studies identified cottonseed and clover feeding as reducing fecal excretion of *E. coli* O157:H7 in dairy cattle (Garber et al., 1999). Other studies have demonstrated that gram-negative bacteria are infrequently recovered from dust at feedlots, possibly because of their rapid inactivation by UV irradiation (Wilson et al., 2002). In contrast, animals housed indoors may be exposed to gram-negative bacteria on dust particles (Zucker et al., 2000). A greater prevalence of *E. coli* O157:H7 was reported (2005) in dairy cows housed on sawdust bedding compared with herds bedded with sand (P = 0.05). Clearly, given the diverse management difference between beef and dairy cattle, preharvest control measures in these 2 production systems will probably differ significantly.

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1995; Dargatz et al., 1997). However, other studies reported a positive association between cottonseed in the diet of feedlot cattle and E. coli O157:H7 prevalence (Sargeant et al., 2004). Likewise, corn silage, barley, and beet pulp appear to increase the carriage rates of E. coli O157:H7 in cattle (Dargatz et al., 1997; Herriott et al., 1998). In a commercial-scale field trial, Berg et al. (2004) demonstrated that feedlot cattle fed barley had a greater prevalence and magnitude of E. coli O157:H7 than corn-fed cattle. A controversial study on the subject of E. coli and feed components, specifically high concentrate diets and forage-rich diets, in cattle appeared in Science in 1998 (Diez-Gonzalez et al.). The authors’ claim that briefly switching cattle from a grain-fed diet to hay shortly before slaughter would reduce the number of cattle positive for E. coli O157:H7 entering the food chain prompted numerous investigations on the subject, none of which provided convincing evidence of predictable or repeatable results (Hovde et al., 1999; Buchko et al., 2000; Callaway et al., 2003).

There are many biologically plausible explanations for how a particular feed might influence the gastrointestinal microbiota (e.g., altering volatile fatty acid concentrations, changing the pH conditions, and altering the composition of the resident bacteria). Our current understanding of the complex mechanisms involving maintenance of the microbial ecosystem in the digestive tract of cattle and E. coli O157:H7’s response to this change in environment, however, are inadequate to predict with reasonable certainty the impact of specific feed components on E. coli O157:H7. Until such time that these interactions are better understood, selection of particular feeds to control E. coli O157:H7 in live animals will be a hit-and-miss process, which will require multiple large, expensive trials to validate.

**Probiotics**

Probiotics are defined as “a preparation of or a product containing viable, defined microorganisms in sufficient numbers, which alter the microflora in a compartment of the host and that exert beneficial health effects in this host” (Schrezenmeir and de Vrese, 2001). These preparations are also called direct fed microbials. Direct fed microbial preparations generally consist of individual bacterial species or mixtures of bacterial species and yeast, often including lactic acid bacteria. Direct fed microbials have been used in the cattle industry for over 20 yr to enhance animal health and production (Nocek and Kautz, 2006). More recently, interest has focused on the potential of these products to affect foodborne pathogen carriage in animals (Tourout, 1989; Brashears et al., 2003; Younts-Dahl et al., 2005). Researchers have isolated several E. coli strains from cattle capable of producing colicins (proteins that specifically inhibit E. coli) that show potential to displace E. coli O157:H7 in live cattle (Zhao et al., 2003) and reduce fecal excretion of enterohemorrhagic Escherichia coli in neonatal calves (Zhao et al., 2003) and in weaned calves also (Tkalcic et al., 2003). This culture’s effectiveness in preventing E. coli O157:H7 colonization under commercial conditions has yet to be examined. Another product, a Lactobacillus acidophilus culture, has repeatedly demonstrated effectiveness at reducing E. coli O157:H7 in feedlot cattle by up to 50% (Brashears et al., 2003; Elam et al., 2003; Younts-Dahl et al., 2005). This product is currently available commercially in the United States and is being used in many, if not most, large US feedlots (Callaway et al., 2004).

**Prebiotics**

Prebiotics are organic compounds such as fructo-oligosaccharides that are unavailable to, or indigestible by, the host animal, but are digestible by a specific bacterial species (Schrezenmeir and de Vrese, 2001). The use of prebiotics has been used in humans to promote intestinal health, and in pigs to improve nutrition, but little information is available on the use of these products in cattle (de Vaux et al., 2002). A preliminary report by Braden et al. (2004) described an inhibitory effect of brown seaweed (Ascophyllum nodosum) on E. coli O157:H7 in feedlot cattle. However, comparing prevalence values among multiple pens of animals is required to further elucidate the effect of this product, if any, on bovine E. coli O157:H7 prevalence.

**DIRECT ANTIPATHOGEN STRATEGIES**

The goal of direct antipathogen strategies is to specifically target and kill pathogenic bacteria through the use of hide washing, antimicrobials, bacteriophages, manipulation of animal physiology with selected compounds, or vaccination.

**Hide Washing**

This approach involves physical removal of contaminants from the hide and hooves from the cattle immediately before, or just after, slaughter. The initial research indicates that such interventions have significant impacts on carcass contamination (Bosilevac et al., 2005a). Alternatives that have been evaluated include wash water or treated water (ozonated or electrolyzed; Bosilevac et al., 2005b). However, such practices should be weighed against humane handling concerns, food quality issues (e.g., increased dark cutters), and even a potential increase in contamination from slaughtering animals with wet hides.

**Antimicrobial Compounds**

For unknown reasons, E. coli O157:H7 does not typically exhibit resistance to the large number of antibiotics frequently observed in other enteropathogenic E. coli and other foodborne pathogens, such as Salmonella enterica. Most isolates are susceptible to the antibiotic, neomycin sulfate (Galland et al., 2001; Stephan and
Schumacher, 2001; Mora et al., 2005). Neomycin sulfate has been demonstrated to decrease fecal populations and excretion of \textit{E. coli} O157:H7 in cattle (Elder et al., 2003; Lefebvre et al., 2006). Neomycin is a good candidate for use in the cattle industry because of its approved use and 24-h withdrawal; however, it is closely related to other antibiotics from the same aminoglycoside family (e.g., streptomycin, kanamycin, and gentamycin) that are used to treat some human infections. Because the risk associated with antimicrobial resistance in human health, its use in cattle remains controversial. The use of ionophores does not appear to significantly influence the prevalence of \textit{E. coli} O157:H7 in cattle (Edrington et al., 2003; Lefebvre et al., 2006).

A nonantibiotic alternative for the selective killing of \textit{E. coli} O157:H7 and other \textit{Enterobacteriaceae}, including \textit{Salmonella}, is the use of sodium chlorate. When administered in feed and drinking water, it reduces \textit{E. coli} O157:H7 populations in the feces and in the intestinal content of cattle (Callaway et al., 2002) and pigs (Anderson et al., 2001) under experimental conditions. The beneficial effects of sodium chlorate are ascribed to the anaerobic reduction of this chemical by nitrate reductase to chlorite, a bactericidal metabolite. Treatment with sodium chlorate is suggested as a perharvest intervention to be applied shortly before slaughter; nevertheless, the impacts on beneficial rumen microflora appear to be negligible (Anderson et al., 2000). The use of sodium chlorate in cattle and other food animals has not yet been approved for use in the United States.

\textbf{Bacteriophage Therapy}

Bacteria are subject to lysis and killing by a large number of viruses called bacteriophages, many of which are commonly found in the intestinal microbial flora of food-producing animals and also in the environment (Klieve and Bauchop, 1988; Klieve and Swain, 1993). Some bacteriophages have fairly narrow host ranges (Barrow and Soothill, 1997; Barrow et al., 1998) and therefore have been studied as potential therapeutic agents for the selective elimination of specific pathogens from a mixed microbial populations, such as those present in the gastrointestinal tract of live animals. Several in vitro studies show remarkable effects of bacteriophages on \textit{E. coli} O157:H7. The principle of phage therapy to combat \textit{E. coli} O157:H7 in cattle is further supported by in vivo work in murine models (Tanzi et al., 2005; Sheng et al., 2006). Unfortunately, the effectiveness of bacteriophage treatment to decrease \textit{E. coli} O157:H7 in cattle or other ruminants has not been frequently reported in the peer-reviewed literature. The few available reports demonstrate the potential for the use of this technology to reduce \textit{E. coli} O157:H7 carriage in cattle, but commercial application of bacteriophage therapy is likely still several years away (Sheng et al., 2006). Widespread use of bacteriophages for treatment of foodborne pathogens in animals will require both regulatory approval and consumer acceptance.

\textbf{Vaccination}

The use of vaccination to prevent pathogen colonization and fecal excretion in agricultural animals is based on the priming of the animal’s immune system against antigens expressed by \textit{E. coli} O157:H7 to prevent the colonization of this organism in the gastrointestinal tract. Achieving these goals is not without challenges; specifically, priming the mucosal immune system to mount a protective response against an otherwise commensal organism has been difficult. Nonetheless, researchers have developed experimental vaccines against the proteins and other cellular components thought to play critical roles in bacterial adherence to mammalian cells and the intestinal mucosa of calves and piglets. These include the Type III \textit{E. coli}-secreted proteins, Tir, intimin, and the O157 lipopolysaccharide (Konadu et al., 1999; Dean-Nystrom et al., 2002; Potter et al., 2004). Preliminary results (Potter et al., 2004) were deemed to be encouraging enough to warrant further investigation; however, the reasons why similar decreases in \textit{E. coli} O157:H7 were not observed among vaccinated cattle in a larger, commercial-scale study involving 218 pens of feedlot cattle in 9 feedlots in Canada remain unanswered (Van Donkersgoed et al., 2005). In the future, it may be possible to engineer effective vaccines, including ones that can be delivered in the feed (Judge et al., 2004; Wen et al., 2006); however, at present, there is no vaccine that is available to reduce \textit{E. coli} O157 in cattle.

In summary, because of the ecology of \textit{E. coli} O157, notably its ability to colonize multiple animal hosts and to survive extended periods in the environment, complete eradication of the organism from cattle populations is unlikely. Nevertheless, control of this foodborne pathogen on the farm may be achieved by restricting the dissemination of pathogens between farms and by limiting proliferation, survival, and dissemination of pathogens within a farm. Considerable efforts have been made in identifying plausible herd management practices and environmental factors that may reduce the prevalence and magnitude of \textit{E. coli} O157 in cattle. Preharvest interventions should be economical, practical, and suitable from an animal welfare perspective.

The reasons that most of the interventions attempted to date have not been successful are unknown. It is possible that the desired goal was not adequately achieved to test the hypothesis because of scientific or practical limitations. For example, it is possible that we have been unable to consistently deliver sterile feed or water to cattle or induce a strong mucosal immune response to a vaccine that could affect \textit{E. coli} O157 carriage. An alternative explanation is that the mechanisms we are trying to interrupt are insignificant in the larger picture of total \textit{E. coli} O157 exposure and...
colonization mechanisms. Although good agricultural practices or good management practices may have an impact on animal health, productivity, and environmental sustainability, unless they are specifically validated with respect to the biological contribution (attributable risk) that each of these factors has on pathogen reduction, they should not be viewed as critical control points for *E. coli* O157:H7 prevention.

**SUMMARY AND CONCLUSIONS**

Currently, only 1 preharvest control for *E. coli* O157:H7 in cattle has been repeatedly shown to be effective for use in reducing the prevalence of *E. coli* O157:H7 in cattle—the probiotic *Lactobacillus acidophilus*. Moreover, because of the potential economic benefits of discovering effective preharvest interventions, it is possible that progress beyond what is described in this review has been made but is not publicly available. Future research into the effectiveness of parallel and simultaneous application of one or more preharvest control strategies, as well as the identification of new preharvest control techniques, may provide a practical means to substantially reduce the incidence of human *E. coli* O157-related illness by intervening at the farm level.

**LITERATURE CITED**


