



# Pre-Harvest Control of *E. coli* O157:H7

## PRE-HARVEST MANAGEMENT CONTROLS AND INTERVENTION OPTIONS FOR REDUCING *ESCHERICHIA COLI* O157:H7 SHEDDING IN CATTLE

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### ABSTRACT

Cattle can be naturally colonized with enterohemorrhagic *E. coli* (EHEC; also known as Shiga toxin-producing *E. coli*, STEC) in their gastrointestinal tract. In order to further curtail human illness and ensure a safe and wholesome food supply, research into pre-harvest *E. coli* O157:H7 and non-O157:H7 EHEC reduction controls and interventions has grown in recent years. This review addresses the controls and interventions that have been proposed or implemented to reduce EHEC in cattle. The interventions are divided into 3 broad categories: 1) management practices and transport, 2) cattle water and feed management, and 3) live animal treatments. Pre-harvest strategies do not eliminate the need for good sanitation procedures in the processing plant or during food preparation. Live-animal interventions to reduce pathogens must be installed in a multiple-hurdle approach that complements in-plant interventions, so reduction in pathogen entry to the food supply can be maximized. Recently, an increase in the research focused on developing new interventions (e.g., vaccination, DFM, chlorate, phages) and on understanding what effect diet and the microbial population has on EHEC populations in the gut of cattle has been observed. This research has resulted in several novel interventions and potential dietary additions or changes that can reduce EHEC in cattle, including those in, or very near to entering, the marketplace.

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# WHITE PAPER

## Beef Safety

## EXECUTIVE SUMMARY

Cattle can be naturally colonized with enterohemorrhagic *E. coli* (EHEC; such as *E. coli* O157:H7) in their gastrointestinal tract. This bacteria can cause serious illness and sometimes death in humans who become infected via food, water, or direct animal contact. To reduce potential human illness and ensure a safe and wholesome food supply, pre-harvest *E. coli* O157:H7 and non-O157:H7 EHEC control and intervention development has occurred rapidly in recent years. The proposed live cattle pathogen-reduction intervention or control strategies can be divided into three broad categories: 1) management practices and transport, 2) cattle water and feed management, and 3) live animal treatments.

### Management Practices and Transportation

Good management of cattle is critical for efficient animal production, but to date, no typical “management” procedures have been shown to affect colonization or *E. coli* O157:H7 shedding. *E. coli* O157:H7 can survive for lengthy periods in manure, soil, and other organic materials. Overall, cleaning bedding or pens will not eliminate *E. coli* O157:H7 from any farm or feedlot environment, but it may slow spread within a group of cattle. Biosecurity efforts are probably minimal in their direct impact on food safety on the farm, simply reducing ways for pathogens to move between “clean” groups of cattle. Reduced animal density can reduce contamination. Since cattle can be exposed during transport and in holding facilities prior to processing, cleaning of trailers and facilities can help reduce the spread of EHEC.

### Cattle Water and Feed Management

Water troughs can contain *E. coli* O157:H7 for long periods of time, and therefore, can facilitate pathogen transmission. Chlorination and cleaning of troughs can reduce EHEC populations and transmission, but limitations to its effectiveness have been seen. Fasting can increase the shedding of *E. coli* O157:H7, as can certain feeds, such as barley and distiller’s grains, or some other form of grain. Steam flaking of grain produces higher *E. coli* O157:H7 populations than does dry rolled grain. Forage-fed cattle

generally contain lower *E. coli* O157:H7 populations, but this is not a complete cure. A sudden shift in diet from grain to hay generally causes a decrease in *E. coli* O157:H7, but the role of the forage quality and type on reduction is currently unknown. Direct-fed microbials, organic acids, seaweed products, and citrus pulp and peels also hold promise as dietary additions to reduce *E. coli* O157:H7 in cattle. These products are currently in the market, but have not been examined in large-scale trials to determine their effectiveness.

### Live Animal Treatments

Bacterial viruses (bacteriophage) can be used to specifically target *E. coli* O157:H7 in cattle. Phages have been approved and marketed for use as a hide spray to reduce the entry of *E. coli* O157:H7 on the hides of cattle entering the food chain. Hide washes have been implemented in many processing plants using organic acids or ozonated water to reduce *E. coli* O157:H7 on the hides of cattle entering the processing facility. Vaccines also have been developed using several avenues of attack to reduce *E. coli* O157:H7 in cattle. Currently, vaccines have had variable results depending on the number of vaccinations required to elicit fullest immunity. Sodium chlorate selectively targets bacteria equipped with the enzyme nitrate reductase, which includes *E. coli* O157:H7. This bacterial enzyme converts chlorate to a form which kills the bacteria, but does not affect other bacteria; this treatment has shown promise in reducing *E. coli* O157:H7 in cattle.

Overall, these pre-harvest strategies do not eliminate the need for good sanitation and procedures in the processing plant and during food preparation. Instead, these live-animal interventions to reduce pathogens must be installed in a multiple-hurdle approach that complements in-plant interventions, so the reduction in pathogen entry to the food supply can be maximized. Pre-harvest *E. coli* O157:H7 research has resulted in several novel interventions and potential dietary additions or changes that can reduce EHEC in cattle, and many of them are in, or very near to entering, the marketplace.

## INTRODUCTION

Cattle can be naturally colonized with enterohemorrhagic *E. coli* (EHEC; also known as Shiga toxin-producing *E. coli*, STEC) in their gastrointestinal tract (160). The most well known of these human pathogens is *E. coli* O157:H7 which has as a natural reservoir in ruminant animals such as cattle. EHEC-caused illnesses cost the American economy more than \$1 billion each year in direct and indirect costs (251).

The relatively recent (1982) emergence of *E. coli* O157:H7 makes it seem like this organism is a new arrival in the food chain; however, data indicate this organism is far more ancient (177, 239, 299). Ecological theory suggests the best fitted organism for a particular environment will survive in that environment best (93). Because *E. coli* O157:H7 apparently evolved along with its host, it is uniquely fitted to survive in the gastrointestinal tract of cattle. This organism produces a potent cytotoxin that does not seriously impact its preferred host (cattle) because they lack toxin receptors (228), but this same toxin can cause serious illness in humans colonized by *E. coli* O157:H7 (215). Unfortunately, this means the natural commensal-type relationship between EHEC (including O157 and non-O157) and cattle ensures this organism can be passed on to meat products. Transmission most frequently occurs during summer months, and is linked to the well-known summer increase in the prevalence of *E. coli* O157:H7 in cattle (104, 210, 297), not consumer cooking habits or an increase in consumption. (301).

Since the emergence of EHEC, the beef industry has spent more than \$2 billion dollars to combat EHEC in processing plants (161). Although in-plant strategies have significantly reduced populations of EHEC (and other foodborne pathogens), these processing interventions have not been perfect (15, 30). As a result, more than 175,000 human foodborne cases of EHEC occur each year in the United States (248).

In order to further curtail human illness and ensure a safe and wholesome food supply, research into pre-harvest *E. coli* O157:H7 and non-O157:H7 EHEC reduction controls and interventions has grown in recent years (59, 185, 219, 246). The logic behind reducing EHEC in live cattle is simple: 1) will reduce the burden on the plants thus rendering the in-plant interventions more effective; 2) will reduce horizontal EHEC spread from infected animals (especially in “super-shedders”) in transport and lairage; 3) will reduce the EHEC burden in the environment and wastewater streams; and 4) will reduce the risk to those (often city dwellers) in direct contact with animals via petting zoos and open farms.

This review addresses controls and interventions that have been proposed or implemented to reduce EHEC in cattle. The interventions are divided into 3 broad categories: 1) management practices and transport, 2) cattle water and feed management, and 3) live animal treatments.

## MANAGEMENT PRACTICES AND TRANSPORTATION

Good management of cattle is critical for efficient animal production, but to date, typical “management” procedures have not been shown to affect colonization or shedding of *E. coli* O157:H7 or an EHEC. On the contrary, the use of squeeze chutes to process cattle has been shown to increase the odds of hide contamination with *E. coli* O157 (192). Good management practices are critical to ensuring animal health and welfare. Some practices may even reduce horizontal transmission of EHEC within a herd of cattle (113), despite the lack of evidence regarding impact on food safety (185).

### Pens and Bedding

*E. coli* O157:H7 can live for a long period of time in manure, soil, and other organic materials (155, 195, 302) and can be transmitted successively through their environment (255, 256). Cattle, especially dairy cows, are bedded on materials largely chosen for animal health and welfare reasons. Unfortunately, bedding material can harbor the bacteria responsible for mastitis, as well as foodborne pathogenic bacteria that can be spread among cattle (94, 218, 237, 298). Research has shown urine fosters the growth of *E. coli* O157:H7 on bedding, most likely by providing substrate for growth (94). Modeling research has shown the death rate of *E. coli* O157:H7 is increased (292) when bedding cleaning frequency is increased. Research has also shown sand bedding compared to saw dust reduced the transmission of *E. coli* O157:H7 among dairy cows, resulting in lower populations of *E. coli* O157:H7 (184). It is suspected the difference was due to desiccation or the reduced nutrient availability in sand.

For many years it was assumed that feedlot surfaces were simply uncomposted manure-like, but recent molecular studies have indicated the bacterial communities of feedlot surfaces are complex, yet utterly distinct from fecal bacterial populations (99). This suggests the factors favoring survival in the gastrointestinal tract (anaerobic, warm, dark) do not favor survival on the feedlot surface (aerobic, cooler, sunlit). Surfaces such as pond ash do not impact survival of *E. coli* O157 (38); however, studies and anecdotal evidence indicate a greater number of cattle shed *E. coli* O157:H7 when housed in muddy pen conditions than cattle from normal pen conditions. Thus, the condition

of the pen floor may influence the prevalence of cattle shedding the organism (259). These results indicate that wet pens often allow *E. coli* O157:H7 to survive longer periods than do dry conditions (36, 259). Overall, bedding or pen cleaning will not eliminate *E. coli* O157:H7 from any farm or feedlot environment, but it may slow spread within a herd or between penmates.

## Biosecurity

Farm biosecurity is critical for animal health and welfare, especially in regard to animal diseases, but, to date little impact has been demonstrated on foodborne pathogenic bacteria such as *E. coli* O157:H7. Research has shown other animal species, rodents, insects and birds can carry EHEC at least transiently (47, 124, 236, 298). Mixing sheep and cattle has shown to increase the risk of cattle shedding EHEC (265), and a positive correlation between cattle and sheep density was found, at least in the UK (275). Other diverse factors such as the presence of dogs, pigs, or wild geese on the farm have been linked to an increased risk of *E. coli* O157:H7 shedding (131, 276). However, to date, practical biosecurity procedures that address EHEC transmission on farms or feedlots have not been fully devised.

Ruminant animals other than cattle carry *E. coli* O157:H7 (124, 145, 247), including sheep and deer that often share the same pasture, feed bunks and water supplies (47). Researchers have found flies and other insects on farms can carry EHEC from one location to another (2, 134, 164, 277). Furthermore, wild migratory birds such as starlings (294, 298), cowbirds and egrets (Callaway, unpublished data) can carry EHEC (or other foodborne pathogens) between pens, and even between farms long distances apart. Although probably minimal in their direct impact on food safety on a farm, insects and birds represent vectors for pathogens to move between “clean” groups of cattle or farms.

## Housing

Many farms are closed to entry by animals from other farms to prevent transmission of animal disease. Closed herds prevent the spread of *E. coli* O157:H7 (and other pathogens) from one farm to another (113). However some studies indicate a closed farm does not impact *E. coli* O157:H7 incidence (80). The results of this study suggest *E. coli* O157:H7 should be considered common to groups of feedlot cattle housed together in pens (259). Therefore, keeping groups together throughout their time on a farm or feedlot, without introducing new members to groups, appears to reduce horizontal transmission among animals.

A further benefit of grouping cattle involves the use of age as a segregating factor. Young cattle (especially heifers)

shed more *E. coli* O157:H7 than do older cattle (80, 87, 259). While it is not possible to segregate calves from cows, a potential benefit to keeping same-age groups of calves together as they are transported and enter backgrounding or feedlot operations has shown to prevent horizontal transmission between groups. Off-site rearing of dairy heifers may be an important solution to reducing foodborne pathogens, as has been shown in regard to *Salmonella* (138), and the risk of transmission back to the farm by heifers returning from an off-site facility was found to be low (102).

Animal density may also play a role in the horizontal spread of *E. coli* O157:H7 and other foodborne pathogens (290). Densely packed animals have a greater chance of contamination with fecal spread. However, increased animal density reduces the physical footprint and may allow for easier waste handling. Higher animal density can be linked to an increased risk of carriage of some EHEC, including O157:H7 (122, 290). European studies show an association between human EHEC illnesses and animal density (126, 137), though Canadian researchers found a variable impact (224). Further studies found increased stocking density increased shedding of EHEC, independent of group size (265, 274).

The issue of “super-shedders” complicates research into effects of animal density and pathogen shedding (20, 77, 180, 267). If super-shedders exist long term, rather than simply being a transient phase of infection, then interactive effects of animal density and pathogen density exist in the animal that must be accounted for (193, 194). The role of super-shedding animals (even if a transient phenomenon) cannot be discounted in the contamination of hides during transport and lairage, especially in dense conditions (19, 20).

## Transportation and Lairage

Handling and transport to processing plants, feedlots or other farms causes stress (see below) and may spread *E. coli* O157:H7 due to physical contact or fecal contamination, and trailers used may spread pathogens among lots or loads of cattle (192). Studies indicate transport did not affect EHEC populations in cattle; however, in these studies, *E. coli* O157:H7 populations were initially very low (29, 200, 233, 253). On the contrary, other studies found transport caused an increase in fecal shedding of *E. coli* O157:H7 (23). Researchers found cattle transported more than 100 miles had a doubled risk of having positive hides at slaughter compared to cattle shipped a short distance. The question of time in close-confinement versus being in transit was not examined (96). In another study, longer transport times were correlated with increased levels of fecal shedding of *E. coli* O157:H7



(23). A more recent study demonstrated a combination of transport and lairage did not lead to an increase in the number or prevalence of *E. coli* O157:H7 from cattle (114). The presence of a high-shedding animal in a trailer has shown to increase the odds of other animals within the load being hide-positive for *E. coli* O157:H7 (19, 20, 120).

Cattle trailers can be important vectors of *E. coli* O157:H7 to uninfected cattle and are frequently positive for *E. coli* O157:H7 when sampled (29, 88, 233). The incidence of *E. coli* O157:H7 in transport trailers increases the risk of transmission to farms and feedlots through the cattle on these trailers (88). However, to date, the washing of trailers has only been shown to be effective against *Salmonella* contamination in swine (229), yet it is an intuitive solution to prevent some degree of cross-contamination of cattle during a stressful period.

Lairage and holding facilities are further locations that can impact the prevalence and concentration of *E. coli* O157:H7 on hides of cattle, which is an important route of entry to the food supply (15). Studies show the transfer of *E. coli* O157:H7 to hides in lairage at processing plants accounted for more of the hide and carcass contamination than did the population of cattle leaving the feedlot (16). Furthermore, the presence of super-shedding cattle in these pens can increase the cross-transmission of *E. coli* O157:H7 among animals from different farms or feedlots (19, 77). The exact role of lairage and transport in the spread of *E. coli* O157:H7 (and other pathogens) among cattle is unclear, and is likely dependent on time and animal density and may be affected by stress.

## Stress

While humans understand stress intuitively, the discussion of “stress” in animals is fraught with anthropomorphism and complexity. Long-term stress may depress immune function in cattle (75, 165, 244), making them more susceptible to colonization, but short-term effects of stress from weaning, handling or transport on immune status are unknown. To date, the effect of stress on colonization or shedding of *E. coli* O157:H7 is unclear.

Weaning is stressful to calves and has been shown to increase colonization with EHEC (139) and *E. coli* O157:H7 (79). In other studies, however, researchers demonstrated weaning does not affect the likelihood of shedding (276). Interestingly, calving was seen to reduce the possibility of *E. coli* O157:H7 shedding (276). Other stresses such as movement have been identified as playing a role in *E. coli* O157:H7 shedding in Scottish cattle (79), but has not been shown to impact shedding in U.S. cattle systems.

When calves were pre-conditioned to transport stress, they were less susceptible to infection from the environment than were calves not pre-conditioned to this stressor (23). Cattle with excitable temperaments were less likely to shed *E. coli* O157:H7 than were “calm” cattle (51, 253). In pigs, it was found the social stress/excitement of mixing penmates increased fecal shedding of *Salmonella* (73), but this has not yet been shown in cattle. This however, implies a potential role of social stress in cattle during lairage.

Heat stress (and methods to combat it) can have effects on animal health and productivity (52), as well as shedding of *E. coli* O157:H7 and *Salmonella* (73). Water sprinkling to alleviate heat stress in cattle increased measures of animal well-being and decreased *E. coli* O157:H7 populations on the hides of cattle, but did not affect fecal populations. In another study with dairy cattle, researchers found heat stress had no impact on EHEC shedding, but *Salmonella* shedding was increased (110). Further research found heat stress did not impact *E. coli* O157:H7 shedding in cattle (51).

## CATTLE WATER AND FEED MANAGEMENT

Diet and water supplies can be used to reduce horizontal transmission of EHEC among animals on the same farm or in the same feedlot pen. The underlying biology behind this effect remains unclear, but it has been suggested the reduction could be due to increased fecal pH or intermediate end products of the yeast fermentation (e.g., vitamins, organic acids, L-lactic acid). However, these suggestions remain hypotheses (297). While the magnitude of dietary effects is relatively small, it underlines the point that dietary composition can potentially significantly impact *E. coli* O157:H7 populations in the gut of cattle.

### Drinking Water Treatments

Cattle water troughs can harbor *E. coli* O157:H7 for long periods of time (134, 181, 182, 206, 298), and as many as 25% of water samples on cattle farms have been shown to contain *E. coli* O157:H7 (245). These results suggest common-use troughs can be vectors for horizontal transmission of *E. coli* O157:H7 within a group of animals. Organic material in water troughs tends to harbor and protect the EHEC, and modeling research has shown an increase in water trough cleaning frequency would increase the death rate of *E. coli* O157:H7 (292). Chlorination of water supplies is used to reduce bacterial populations in municipal water supplies and can also be used in cattle water troughs to reduce *E. coli* O157:H7 populations. However, sunlight and organic material in the water reduces the effectiveness of chlorination over time, as seen in real world chlorination studies with cattle water troughs

(183). Electrolyzed oxidizing (EO) water has been shown to reduce EHEC populations as high as 104 CFU/ml (271) and can be used as an in-plant hide cleaning strategy (43). Adding treatments such as cinnamaldehyde and sodium caprylate to water supplies has proven to reduce EHEC populations, but the effects on palatability are not currently known (5, 78).

## Fasting

Cattle can be fasted up to 48 h before and during their transport to slaughter, which can affect the prevalence of *E. coli* O157:H7. Ruminal and intestinal volatile fatty acid (VFA) concentrations limit the proliferation of *E. coli* due to the toxicity of the VFA to the bacteria (140a, 242, 303). This led to the use of organic acids/VFA as a method to alter the ruminal fermentation and reduce pathogen populations in the gut (217, 227, 287). However, fasting causes levels of VFA to decline rapidly (135).

Fasting increased *E. coli*, *Enterobacter* and total anaerobic bacterial populations throughout the intestinal tract of cattle (55, 130), and increased *Salmonella* and *E. coli* populations in the rumen (53, 129). Research has demonstrated fasting can cause “apparent *E. coli* (O157:H7)- negative animals to become positive” (172). Fasting made calves more susceptible to colonization when inoculated with *E. coli* O157:H7 (86). Cattle fasted for 48 h prior to slaughter contained significantly larger *E. coli* populations throughout the gut than cattle fed forage (130). In contrast, the fasting period had no effect on *E. coli* O157:H7 shedding (135). In general, studies examining the intestinal environment have repeatedly indicated low pH and high concentrations of short-chain VFA result in lower EHEC populations (22, 27, 81, 257). Thus, most research supports the concept that fasting increases shedding or population size, or can make cattle more susceptible to colonization due to decreased short-chain VFA and increased pH in the gastrointestinal tract. Because feed withdrawal and/or starvation results in decreased VFA concentrations in the gut, it has been suggested that this shift plays a role in the effects of transport and/or lairage on the shedding of EHEC.

## Feed Types

The first dietary practice to significantly increase the risk of EHEC shedding among heifers was feeding corn silage (139). In adult cows, the inclusion of animal by-products in the diet (currently discontinued) was also shown to increase EHEC shedding (139). Other studies linked feeding whole cottonseed with reduced *E. coli* O157 shedding (128, 133). Fecal samples from cattle fed a controlled diet of dry-rolled corn, high-moisture corn and wet corn gluten feed did not contain different populations of generic *E. coli*, or extreme acid-resistant *E. coli* (254). However, feces from cattle

free-fed wet corn gluten contained significantly higher concentrations of extreme acid-resistant *E. coli* (resistant to an acid shock simulating passage through the human stomach) than did feces of cattle free-fed dry-rolled or high moisture corn (254).

Barley is often fed to cattle and is ruminally fermented more rapidly than corn by the commensal microbial population. More starch is fermented in the lower gut of corn-fed cattle than in barley-fed cattle, resulting in barley-fed cattle having higher fecal pH and lower VFA concentrations compared with corn-fed animals (26, 34, 54). Feeding Barley has been linked (albeit at a low correlation) to increased *E. coli* O157:H7 shedding (92), and, in experimental infection studies, barley feeding was again associated with increased shedding of *E. coli* O157:H7 by feedlot cattle (54). Survival of *E. coli* O157:H7 in manure from corn- and barley-fed cattle is approximately equal; therefore, survival in the feces is not the reason for increased prevalence of *E. coli* O157:H7 in barley-fed cattle (27).

## Distiller's Grains

The industrial fermentation of corn to produce ethanol has increased more than 4-fold between 2001 and 2007, and ethanol use doubled by 2010 (238). Thus, an economic incentive to increase the utilization of distiller's grains (DG) by-product feeds in the cattle industry has increased dramatically in recent years, especially given the role of DG as a cost-effective feed supplement for finishing and lactating cattle (115). The inclusion of DG into cattle rations is an effective replacement for common feedstuffs and has demonstrated an increased daily gain in beef cattle (3) and milk yield and feed efficiency in dairy cows (170), potentially by altering the microbial ecosystem of the rumen (65).

Unfortunately, research suggests a potential association between feeding dry distiller's grains with solubles (DDGS) and an increased prevalence and fecal shedding of *E. coli* O157:H7 in cattle (150, 151, 304). Distiller's grains were shown to increase the shedding of *E. coli* O157:H7 in cow-calf operations in Scotland (276). Other research found feeding a related product (brewer's grain) to cattle was also associated with increased *E. coli* O157 shedding, and increased the odds of shedding by more than 6-fold (95). Individual animal prevalence of feedlot cattle shedding *E. coli* O157 on d 122 (but not d 136) was higher in cattle fed 25% wet distiller's grain (WDG) compared to control diets lacking WDG (151), but pen-level shedding was unaffected by WDG feeding.

Pen-floor fecal-sample prevalence of *E. coli* O157 was significantly higher across a 12-week finishing period in cattle fed 25% dry distiller's grain (DDG) and either 15%

or 5% corn silage compared with cattle fed 0% DDG and 15% corn silage (150). However, follow-up studies found no differences in *E. coli* O157:H7 fecal shedding in cattle fed DG (109, 149), with no indication of why a difference in results was observed. In a further study, utilizing both dry and wet distiller's grains, researchers found higher levels (40% of the ration) of DG inclusion did increase fecal *E. coli* O157:H7 shedding (153). When cattle were fed 40% WDG, higher populations of *E. coli* O157:H7, higher pH values and lower concentrations of L-lactate were observed (297). Interestingly, researchers found the numbers of *E. coli* O157:H7 were greater in fecal *in vitro* incubations containing corn DG than wheat DG (304).

### Grain Form

Research has shown the form of corn included in cattle rations can impact *E. coli* O157:H7. In feedlot cattle, steam-flaked grains increased *E. coli* O157 shedding in the feces compared to diets composed of dry-rolled grains (118). This difference is theorized to be because dry rolling allows the passage of more starch to the hindgut where it becomes fermented to produce VFA, thereby killing *E. coli* O157 (118). This theory is supported by the fact that post-ruminal starch infusion numerically increased generic *E. coli* populations in the lower gut (288).

### Forage Feeding

*Escherichia coli* thrives in the lower gut of animals fed forage diets (146, 147, 154). When comparing grain-fed to forage-fed cattle, more *E. coli* (including O157:H7) are present in the feces of cattle fed grain diets. The effects of high-grain or high-forage diets on the duration of shedding or fecal *E. coli* O157:H7 populations in experimentally inoculated calves have been examined. In these studies, calves that consistently shed the highest concentrations of *E. coli* O157:H7 were fed a high-concentrate (grain) diet (282). However, ruminal fluid collected from steers fed a high-forage diet allowed *E. coli* O157:H7 to proliferate to higher populations *in vitro* than did ruminal fluid from high-grain-fed steers (282), possibly due to differences in VFA concentrations between the ruminal fluids.

Other research found feeding forage actually increased the shedding of *E. coli* O157:H7 in cattle (285). When cattle were fed forage, *E. coli* O157:H7 was shed for 60 days compared to 16 days, for cattle on a grain-based diet (285). Studies examining the effects of forage on survival of *E. coli* O157:H7 in manure found low-quality forages caused a faster rate of death of *E. coli* O157 populations (123) indicating a possible role of forage chemical or secondary plant components (such as tannins, see page 8) in fecal shedding (199). Other studies found feeding forage-rich secondary compounds, such as sainfoin, might be a method to manipulate fecal populations of *E. coli* O157:H7 to a

limited extent (33).

Although *E. coli* O157:H7 populations are generally lower in cattle fed forage diets, it must be emphasized that EHEC are still isolated from cattle solely fed forage, so forage feeding is not a magic bullet (147, 281). Many outlets have claimed grass-fed cattle contain fewer pathogens than do cattle fed grain; however, this has not been demonstrated scientifically. Researchers have found no difference in food safety parameters of beef from grass-fed cattle versus grain-fed cattle (308). Furthermore, research into organic versus conventional rearing systems have demonstrated no difference in the incidence of *E. coli* O157:H7 shedding (152, 234).

### Dietary Shifts

A sudden shift from grain to hay appears to cause a severe, widespread disruption in the gut microbial flora population. Thus, the effects of rapid dietary shifts on the microbial population in regards to *E. coli* O157:H7 populations have been examined. Early studies investigating (generic) *E. coli* and dietary effects indicated a sudden decrease in hay intake by cattle increased fecal *E. coli* populations (53). Other studies using experimentally infected sheep found a sudden switch from an alfalfa pellet diet or a corn/alfalfa ration to a poor-quality forage diet increased *E. coli* O157:H7 shedding (172, 173).

Cattle fed feedlot-type rations contained (generic) *E. coli* populations that were 1000-fold higher than cattle fed a 100% good-quality hay diet (98). When abruptly switched from a 90% grain-finishing ration to a 100% hay diet, fecal *E. coli* populations declined 1000-fold within 5 days (98). However, important to note in this study; *E. coli* O157:H7 was not detected. Based on these results, the authors suggested feedlot cattle could be switched from high-grain diets to hay for 5 days prior to slaughter to reduce *E. coli* contamination entering the abattoir (98). Research indicates a brief (5 d) period of hay feeding does not impact carcass characteristics; however, if the hay feeding takes place during the final portion of the finishing period, it causes lower dry matter intake (DMI) and loss of 2.2 lb/head/d (269). Although not as large a reduction as reported by Diez-Gonzalez et al (1998), feeding hay significantly reduced total coliform counts and (generic) *E. coli* counts (269), without a significant impact on carcass weight, dressing percentage, carcass grade, or quality parameters. Cattle fed hay for a 48 h period immediately prior to transport to slaughter did not lose more weight during transport than fasted or pasture-fed animals (130).

A group of cattle identified to naturally carry an *E. coli* O157:H7 infection was divided into two groups, one fed grain and the other abruptly switched to hay. Fifty-two



percent of the grain-fed controls remained *E. coli* O157:H7 positive, but only 18% of the hay-fed cattle continued to shed *E. coli* O157:H7. This switch resulted in a body weight (BW) decrease of 1.25 lb/hd/d compared to the controls (163). Other research found cattle fed a high-concentrate diet then switched to a hay diet containing 50/50% corn silage/alfalfa had lower *E. coli* counts (0.3 log10) after 4 days (158). Cattle fed an 80% barley ration, fasted for 48 h and then subsequently switched to 100% alfalfa silage, did not exhibit any change in *E. coli* O157:H7 shedding (55). When these same animals again fasted for 48 h and then re-fed alfalfa silage, the prevalence of *E. coli* O157:H7 shedding increased significantly (55). Experimentally infected cattle fed hay shed *E. coli* O157:H7 significantly longer than grain-fed cattle (42 d vs. 4 d), however, the *E. coli* O157:H7 populations shed were similar between diets (142). Cattle abruptly switched from a finishing diet containing wet corn-gluten feed to alfalfa hay for 5 d, had an increased colonic pH and an approximate 10-fold decrease in total *E. coli* populations (254).

Conversely, when cattle were switched from a forage-type diet to a high grain-finishing ration, fecal and ruminal generic *E. coli* concentrations increased (37). In another study, slightly outside of the “normal” dietary switch structure, cattle switched from pasture to hay 48 h prior to slaughter had significantly reduced *E. coli* populations throughout the gut (130). Gregory et al., found feeding hay increased intestinal Enterococci populations capable of inhibiting *E. coli* populations similar to a competitive exclusion (CE) culture. However, in this New Zealand-based study, the effects of high-grain versus forage diets were not examined (130). Based on the data, the authors concluded, “the most effective way of manipulating gastrointestinal counts of *E. coli* was to feed hay” (130).

Collectively, these results emphasize dietary manipulations such as shifting cattle from a high-grain to forage ration prior to harvest could be a powerful method to reduce *E. coli*/EHEC populations in cattle. However, the mechanism remains unknown, and the effect is inconsistent. It appears a factor contributing to inconsistency involves forage quality and type, but this remains a hypothesis. The presence of end products of fermentation (e.g., VFA) as well as some secondary compounds in forages, may play a role in pathogen populations and prevalence. While a dietary switch to forage in feedlots is not advocated due to feasibility, weight loss and other logistical issues, other high fiber feedstuffs (e.g., soy hulls, cottonseed meal) or feedstuffs rich in phenolics or essential oils (see next section), may be a more feasible alternative strategy to decrease *E. coli* O157:H7 populations.

## Tannins, Phenolics, and Essential Oils

Plants contain phenolic compounds, such as lignin and tannins, that may affect the microbial ecosystem of the gastrointestinal tract through antimicrobial action (33, 85, 143, 149, 221). Tannins have been demonstrated to significantly inhibit the growth of *E. coli* O157:H7 *in vitro* and generic *E. coli* populations in cattle (33, 89, 199, 295). Other researchers found phenolic acids that comprise lignin also demonstrate antimicrobial activity against *E. coli* O157:H7 in fecal slurries. Highly lignified forages show a reduced period of *E. coli* O157:H7 shedding compared with cattle fed only corn silage (296).

Essential oils are most often associated with aromatic plant compounds used as spices or extracts (28). Many essential oils exhibit antimicrobial activity (100, 116, 167, 222, 232, 284), often through the action of dissolving bacterial membranes (97, 284). As a result, many plant products have been used for centuries for the preservation and extension of the shelf life of foods (91). Essential oils have been proposed as potential modifiers of ruminal fermentation (31, 32, 39, 221) and to reduce *E. coli* O157:H7 in the live animal (31, 149).

## Seaweed (Tasco)

Brown seaweed (Tasco-14) is a feed additive included in cattle diets to improve carcass quality characteristics and shelf life, increase antioxidants and improve ruminal fermentation efficiency (8, 46, 187). *In vitro* studies have indicated Tasco-14 can reduce populations of *E. coli* and *Salmonella* (Callaway, unpublished data). More recent results link this anti-pathogen activity to the presence of phlorotannins in brown seaweed (295). The phlorotannin anti-*E. coli* activity is greater than found in other studies with terrestrial tannin sources (199, 295). Studies *in vivo* found Tasco-14 feeding reduces fecal and hide prevalence of *E. coli* O157 in cattle (45). Although Tasco-14 is currently available in the market place and can be included in cattle rations, the extent of anti-pathogen activity *in vivo* is still not clear; therefore the cost of inclusion must be weighed carefully by the producer.

## Citrus Products

For many years, orange peel and citrus pulp have been included in dairy and beef cattle rations because they have excellent nutritional characteristics for cattle and are low-cost (14). Citrus fruits contain a variety of compounds, including essential oils and phytochemicals that exhibit antimicrobial activity against foodborne pathogens (125, 201, 209, 291). Research demonstrates the addition of > 1% orange peel and pulp to rations reduced populations of



*E. coli* O157:H7 and *Salmonella* Typhimurium in mixed ruminal fluid fermentations in the laboratory (64, 209). Further studies show feeding orange peel and pulp reduces intestinal populations of diarrheagenic *E. coli* in weaned swine (82). In ruminants, feeding orange peel and citrus pellets (a 50/50 mixture) at levels up to 10% DM, reduced artificially inoculated populations of *E. coli* O157:H7 and *Salmonella* Typhimurium in sheep (62, 63). To date, orange peel feeding has not been examined in large-scale feeding studies, but it retains promise as a potential strategy to reduce the burden of pathogens on the farm, thus reducing environmental contamination and re-infection.

## Organic Acids

Organic acids are used in animal nutrition to modify the ruminal fermentation by providing some members of the microbial ecosystem a competitive advantage and inhibiting other species (191, 212, 225). Some organic acids (such as lactate, acetate, propionate, malate) have been shown to have antimicrobial activity against *E. coli* O157:H7 (136, 243, 289, 303). These acids have been used on hide and carcass washes to reduce pathogen populations, but only recently has the use of organic acids to reduce pathogens in live animals received interest (71, 211). Preliminary results show some success in inhibiting pathogens in the lower intestinal tract of animals (unpublished data); however, further research is needed to identify the appropriate organic acid and concentration, and the correct intestinal location to release the organic acid, to reduce populations of *E. coli* O157:H7 in cattle.

## Ractopamine

$\beta$ -agonists, such as ractopamine, are used in cattle to improve animal performance and carcass leanness. *In vitro*, ractopamine had no effect on growth parameters of *E. coli* O157:H7 (106); but when used in sheep, the fecal shedding and cecal populations of *E. coli* O157:H7 were increased (106). When feedlot cattle were fed ractopamine, the number of cattle shedding *E. coli* O157:H7 decreased (105). In a follow-up study,  $\beta$ -agonist (ractopamine and zilpaterol) treatment had an insignificant effect on fecal shedding of *E. coli* O157:H7 in cattle (108). Taken as a whole, these results indicate the effects of  $\beta$ -agonist feeding are minimal or non-existent on *E. coli* O157.

## Ionophores

Ionophores, such as monensin and lasalocid, are antimicrobial compounds included in most feedlot and dairy rations to inhibit gram-positive bacteria, thereby improving feed:gain ratios and production efficiency (70). Because these feed additives affect the gram-positive portion of the microbial population, possibly giving gram-

negative bacteria (such as *E. coli*) a competitive advantage, research has been conducted to identify their role in the spread of *E. coli* O157:H7 in cattle. *E. coli* O157:H7 has a true gram-negative membrane physiology; thus, ionophores did not affect the growth of this pathogen *in vitro* when added at concentrations up to 3-fold higher than those normally found in the rumen (24, 285).

Early studies demonstrated a marginal increase of EHEC shedding by heifers fed ionophores (139), but other studies found no effect (92). Further, a short-term, 12-day study examining the effect of ionophoric feed additives (monensin, lasalocid, laidlomycin and bambarmycin) on *E. coli* O157:H7 in experimentally inoculated lambs demonstrated no effect *in vitro* (107) on fecal shedding or intestinal populations (103). In an *in vivo* study, cattle fed a forage ration including monensin shed *E. coli* O157:H7 for a shorter period of time than forage-fed cattle not supplemented with monensin, but monensin had no effect on shedding when cattle were fed a corn-based ration (285). In an *in vitro* study, monensin and the co-approved antibiotic tylosin (tylan) treatment reduced *E. coli* O157:H7 populations up to 2 log<sub>10</sub> CFU/ml in ruminal fermentations from cows fed forage, but this did not extend to *E. coli* O157:H7 populations in ruminal fluid from cows fed corn (196). Researchers later found the inclusion of monensin and tylosin in a barley (grain)-based diet did not alter fecal shedding of experimentally-inoculated *E. coli* O157:H7 (196). These results suggest a potential interaction between diet and ionophore inclusion may have an effect on *E. coli* O157:H7 populations.

## Antibiotics

The use of antibiotics to specifically control *E. coli* O157:H7 shedding in cattle is controversial. To date, little research has been conducted in this area. Neomycin, an antibiotic approved for use in cattle to treat enteric infections, has been shown to reduce *E. coli* O157:H7 populations in the gut (112, 230) and on the hides of cattle (230). Other researchers found the feeding of chlortetracycline and tylosin decreased fecal shedding in swine artificially infected with *E. coli* O157:H7, while bacitracin did not impact *E. coli* O157:H7 populations (84). It is hypothesized the generalized disruption of the microbial ecosystem caused by antibiotic treatment indirectly affects *E. coli* O157:H7 populations; thus the use of some antibiotics may provide *E. coli* O157:H7 a competitive advantage in the ruminant gastrointestinal tract. The use of antibiotics to reduce *E. coli* O157:H7 in cattle has not been recommended because of concerns relating to the development of antimicrobial resistance.

## Probiotics: dietary approaches harnessing microbial ecology

Recently the use of probiotics (e.g., the utilization of live or dead cultures of microorganisms to alter the microbial population of the gut) has received increased interest as a method to reduce foodborne pathogenic bacteria in cattle. Traditionally, probiotics have been used in cattle to enhance production efficiency of meat and milk (72, 127, 283, 306). However, recent years have seen an increase in the use of these probiotic types: direct-fed microbials (DFM), competitive-exclusion cultures (CE), and prebiotics to reduce *E. coli* O157:H7 populations in cattle.

In general, it appears probiotic products alter the microbial ecology of the gastrointestinal tract through a variety of mechanisms. As the DFM/CE bacteria attach to the surface of the intestinal epithelium, this physical binding can prevent opportunistic pathogens from attaching to the intestinal wall (83, 168). Volatile fatty acids produced by microbial fermentation can be toxic to some bacterial species (303). Other bacterial products (such as ethanol, traditional antibiotics, or colicins/bacteriocins [described below]) are produced by some intestinal bacteria to eliminate competition within the same environmental niche (148). Collectively, these modes of action demonstrate the complexities involved with interrupting the cycle of transmission and colonization of *E. coli* O157:H7 in cattle and emphasize a multiple-hurdle approach using complementary interventions has the greatest chance of improving food safety at the live animal level.

### Direct-Fed Microbials

These products are widely fed to beef and dairy cattle and are typically composed of yeast, fungal or bacterial cultures or end-products of fermentation, and the cultures may be live or dead. The DFM is fed to animals daily to improve the ruminal fermentation and production efficiency (190). Increasingly, companies claim they are beneficial by reducing *E. coli* O157:H7 shedding in cattle. Researchers compared several commercially available growth-enhancement probiotics and yeast products and found feeding probiotics provided no effect in regards to pathogen levels in cattle (162). When a probiotic culture comprised of *Streptococcus bovis* and *Lactobacillus gallinarum* from the rumen of cattle was given to experimentally infected calves, a reduction of *E. coli* O157 shedding was observed, and this decrease was attributed to an increase in VFA concentration in the gut (216). Probiotic products have been developed specifically to reduce *E. coli* O157:H7 shedding in cattle. In sheep, a probiotic containing *S. faecium* or a mixture of *S. faecium*, *L. acidophilus*, *L. casei*, *L. fermentum* and *L. plantarum* significantly reduced fecal shedding of *E. coli* O157:H7; yet, a monoculture of

*Lactobacillus acidophilus* was found to be ineffective (186). A DFM comprised of *Bacillus subtilis* did not affect fecal prevalence or concentration of *E. coli* O157:H7 or have an impact on average daily gain in feedlot cattle (18). Studies also indicate cultures of *Lactobacillus acidilacti* and *Pediococcus* can directly inhibit *E. coli* O157:H7, likely through the production of organic acids and low pH (240).

Other research shows, when fed to feedlot cattle, a DFM *L. acidophilus* culture derived directly from the rumen of cattle, reduced *E. coli* O157:H7 shedding by more than 50% (48-50). In an independent evaluation, this DFM reduced fecal shedding of *E. coli* O157:H7 in cattle from 46% to 13% (230). In a further refinement of this DFM, the *L. acidophilus* cultures were combined with *Propionibacterium freudenreichii* (a propionate-producing commensal intestinal bacteria) and the prevalence of *E. coli* O157:H7 in the feces was reduced from approximately 27% to 16% and reduced the prevalence on hides from 14% to 4% (111, 307). Further work with this DFM has again shown a reduction of *E. coli* O157:H7 and *Salmonella* in feces and on hides (270).

Bovamine, a *Lactobacillus*-based DFM is currently being marketed and widely used in the cattle industry because it has been reported to improve growth efficiency of cattle, at least in a feedlot ration. A single DFM that can effectively reduce *E. coli* O157:H7 populations in cattle and improve production efficiency in all production systems is unlikely. Therefore, alternative DFM cultures selected specifically for each production segment or situation, need to be developed so food safety improvement can occur while economically balancing the cost of its inclusion in cattle rations, thus “paying for” the enhancement of food safety.

### Competitive Exclusion (CE)

Competitive exclusion is another probiotic approach used to eliminate *E. coli* O157:H7 (as well as *Salmonella*) from cattle gastrointestinal tracts (48-50, 310). Competitive exclusion, as a technology, involves the addition of, a (non-pathogenic) bacterial culture (of one or more species) to the intestinal tract to reduce colonization or decrease populations of pathogenic bacteria (127, 214). An established gastrointestinal microbial population makes an animal more resistant to transient opportunistic infections (127). The species best adapted to occupy a particular niche within the intestinal tract will succeed, while pathogenic bacteria are generally viewed as opportunists.

A CE culture should be derived from the animal of interest, thus CE cultures attempt to take advantage of co-evolution of host and microorganism. Depending on the stage of production of the animal (i.e., maturity of the gut), the goal of CE can be the exclusion of pathogens from the naïve

gut of a neonatal animal, or the displacement of an already established pathogenic bacterial population (214). For example, many researchers have isolated commensal (non-pathogenic) *E. coli* strains that show tendencies to reduce *E. coli* O157:H7 populations, at least *in vitro* (119, 235, 309). Researchers used a defined population of multiple commensal *E. coli* strains and found this generic *E. coli* CE culture could displace an established *E. coli* O157:H7 population from calves (309). In a follow up study, calves colonized with the *E. coli* CE product shed less *E. coli* O111:NM and O26:H111 (both EHEC strains isolated from cattle, but the CE product did not reduce *E. coli* O157:H7 (310).

### **Colicins and Colicin-producing *E. coli*:**

Colicins are antimicrobial proteins produced by certain *E. coli* strains that kill or inhibit the growth of other *E. coli* strains (171, 176, 258), including *E. coli* O157:H7 (159, 207, 249). The concept of using colicins as an intervention strategy to kill foodborne pathogens is not new (207), but until recently has been limited by cost to use as treatment on finished meat products (1, 188, 223) or vegetables (208). Recently, the costs of production and purification of colicins was lowered by recombination protein expression work (266). Due to increased availability of colicins, studies in a mouse model have found *E. coli* O157:H7 can be prevented from colonization (178). Recently, specific studies have examined the use of specific colicins against *E. coli* O157:H7 *in vitro* in gastrointestinal simulations (74) and against other *E. coli* *in vivo* (90).

In spite of the seemingly simple addition of a protein (colicin) to animal diets to control *E. coli* O157:H7, studies have indicated the sensitivity of *E. coli* O157:H7 strains to any single colicin can be highly variable (205, 207, 249). Some *E. coli* O157:H7 strains are colicinogenic and produce specific concomitant immunity proteins (205); therefore, these strains of *E. coli* O157:H7 can be resistant to certain added colicins or even a broad category of colicins (4). Thus, if colicins are to be used as a pre-harvest intervention strategy, simultaneous administration of several categories of colicins must occur. Furthermore, if colicins are to be a viable anti-*E. coli* O157:H7 intervention strategy, the proteins must be protected from gastric and intestinal degradation. Researchers have proposed a specific form of DFM/CE, the feeding of colicin-producing *E. coli* in cattle rations (249, 250, 309), as a way of getting colicins into the lower gut of cattle. These strains have been shown to colonize the lower gut of cattle but reduction in concentration of *E. coli* O157 was approximately 2 log<sub>10</sub> CFU/g, not a complete elimination (208).

Due to the complex nature of a ruminant animal gastrointestinal tract, and the long (12-18 month) life

span of cattle going into a feedlot, CE use in cattle as a “one shot” approach may not completely eliminate *E. coli* O157:H7 and other EHEC shedding throughout the lifetime of the animal. So, individual CE for various phases of production cycles or changes (e.g., entry to the feedlot) may need to be developed, or a CE culture established early may be best supplemented by DFM and/or prebiotic feeding (synbiotics, described below).

### **Prebiotics**

Organic compounds unavailable to, or indigestible by the host animal, but digestible by a specific segment of the microbial population are generally classified as “prebiotics” (252, 293). For example, fructo-oligosaccharides are sugars not degraded by intestinal enzymes that can pass down to the cecum and colon to become “colonic food” for the host bacterial population and provide nutrients to the intestinal mucosa (141, 300). Some prebiotics provide a competitive advantage to specific members of the native microflora (e.g., *Bifidobacteria*, *Butyrivibrio*) by helping exclude pathogenic bacteria from the intestine via direct competition for nutrients or binding sites through the production of “blocking factors”, or antimicrobial compounds in a fashion similar to that of CE (311). Coupling the use of CE and prebiotics is known as “synbiotics”, and could yield a synergistic effect in the reduction of foodborne pathogenic bacterial populations in food animals prior to slaughter (40). To date, prebiotics have not been widely implemented in cattle due to their expense, and the ability of ruminal microorganisms to degrade a wide variety of typical prebiotic substrates. However as costs change, their inclusion as part of a synbiotic directed anti-pathogen strategy may become feasible.

## **OTHER LIVE ANIMAL TREATMENTS**

In spite of dietary and probiotic treatments, other potential pathogen-reduction strategies have been developed for use in the live animal. Many of these treatments utilize the host animal, natural members of the microbial ecosystem, or an aspect of pathogen physiology to inhibit pathogen survival.

### **Bacteriophages:**

All bacteria can be infected by bacteriophages (bacterial viruses) that naturally occur in the environment (175, 179), including the intestinal tract of cattle (69). Phages can have very narrow target spectrums, and may only be active against a single bacterial species, or even strain because they target specific receptors on the surface of the bacterium (179). This specificity should allow phages to be used as an anti-pathogen treatment, a kind of “smart bomb” targeting the species we wish to eliminate, without perturbing the overall microbial ecosystem (157). Phages “hijack” a targeted bacterium’s biosynthetic machinery to



produce daughter phages; when intracellular nutrients are depleted, the host bacterium bursts, releasing phages to repeat the process in a fashion similar to a chain reaction. An exponential increase in the number of phages continues as long as target bacteria are present, allowing phages to persist in the environment rather than simply degrade over time as a chemical treatment. However, phage populations are self-limiting; if the targeted bacteria are removed from the environment, then phage populations diminish. One potential drawback to the use of phages is the rapid development of bacterial resistance to a single phage, thus much of the effort has been focused on the development of multi-phage cocktails (278).

Phages have been examined for use in two different methods to reduce *E. coli* O157:H7, within the gut of cattle before slaughter and as a hide or environmental decontaminant. Commercial phage-based anti-*E. coli* O157:H7 focused on the use of lytic phages in hide wash and surface cleansing products; FSIS has issued a letter of no objection to this use of phages. Phage products for use as a hide spray have been released into the marketplace (Omnilytics and Elanco, Finalyse). Company-based research indicates a significant reduction in positive trim samples from cattle sprayed with this product. Processors are finding appropriate critical control points to include phage sprays on carcasses prior to de-hiding and in relation to other hide spray intervention steps to reduce *E. coli* O157:H7 on the hides of cattle as they enter the food chain.

Phages have been used successfully in several *in vivo* research studies examining the effect of phages on diseases that impact animal production efficiency or health (144, 262-264). Bacteriophage treatment reduced enterotoxigenic *E. coli* (ETEC)-induced diarrhea and splenic ETEC colonization in calves (263, 264). Bacteriophages have been used to control foodborne pathogenic bacteria, especially O157:H7 in cattle gastrointestinal tracts (21, 25, 68, 174, 213, 241). Several different phages have been isolated from feedlot cattle (69, 220) and other sources (197) and have been used to reduce *E. coli* O157:H7 strains in artificially infected animals as proofs of concept (21, 68). Naturally phage-infected ruminants have been shown to be more resistant to *E. coli* O157:H7 colonization (231). Commercialization studies for these on-farm products has had mixed results (268), but studies focusing on the development of appropriate, effective multi-phage cocktails are currently underway (Stanford and McAllister, personal communication).

No matter how phages are used, they must be carefully selected based on: 1) action against multiple *E. coli* O157:H7 strains as well as other non-O157 EHEC strains,

2) ability of members of a cocktail to utilize different receptors to minimize resistance development, and 3) being strictly lytic (i.e., does not transfer genetic material) because phage-mediated transfer is the mechanism by which EHEC originally acquired their Shiga toxin genes (44, 177).

## Vaccination

The immune system of animals is a very potent, anti-pathogen mechanism against bacteria pathogenic to the animal. Therefore, if the power of cattle's immune systems can be applied to address foodborne pathogenic bacterial populations, foodborne illness could be reduced. Immunization has worked effectively against pathogenic bacteria, including *E. coli* strains that cause edema disease in pigs and *Salmonella* in poultry (132, 156). Unfortunately, because EHEC/STEC do not cause disease in cattle, the immunostimulation provided by these foodborne pathogens is not as potent, because it appears natural exposure to *E. coli* O157:H7 does not confer protection to the host (132). Thus, vaccine production has specifically targeted aspects of the physiology of *E. coli* O157:H7. Vaccination is widely accepted in the cattle industry, thus it is reasonable to predict that producers will implement this pathogen reduction technique if the vaccine is economically feasible and can be incorporated into existing production systems.

## Siderophore Receptor and Porin (SRP) Protein Vaccines

Siderophores are proteins excreted by bacteria in an effort to obtain iron from its environment, and *E. coli* O157:H7 secretes siderophores in the intestinal tract of cattle. The SRP vaccine targets this protein and disrupts iron transport into the bacterium, resulting in cell death. The EpiTopix™ SRP vaccine has been conditionally approved for use in cattle in the United States and is undergoing additional safety and efficacy tests. Preliminary research results are promising when the vaccine is utilized in a three-dose treatment regimen (280). Other researchers found that vaccination with the SRP vaccine reduced fecal concentrations of *E. coli* O157:H7 in cattle by 98%, but the vaccine did not affect cattle performance (279). Vaccination of cattle with SRP in another study reduced the prevalence of *E. coli* O157:H7 by nearly 50% (121). Recent unpublished research results have shown a two-dose treatment is not as effective, and vaccination of dams and calves can have variable results on the shedding of *E. coli* O157:H7 at the time of harvest.

## Bacterial Extract Vaccines

A vaccine produced from *E. coli* O157:H7 extracts (type III secreted proteins) has been produced as Econiche™. This vaccine has been licensed in Canada and is pending

a conditional license in the United States. Preliminary experimental results indicated this vaccine reduced *E. coli* O157:H7 shedding in feedlot cattle from 23% to less than 9% (203, 226, 286). In an evaluation study, it was demonstrated that vaccination reduced fecal shedding from 46% to 14% (230). Recent studies have shown an experimental three-dose regimen reduced *E. coli* O157:H7 shedding by 65%, but a two-dose system was less effective (204). However, in a follow-up study, a two-dose regimen was shown to reduce rectal colonization by *E. coli* O157:H7 in feedlot cattle (261). The benefits of vaccinating cattle to reduce the number of hides positive for *E. coli* O157:H7 can be lost if treated cattle come in contact with non-vaccinated cattle during transport (260).

While the Econiche vaccine pioneered the use of bacterial extracts, other extract-type vaccines against multiple *E. coli* O157:H7 proteins (e.g., intimin and tir) have been produced which reduce fecal shedding in experimental infection models (198). Further multiple protein vaccines have been developed that reduce fecal shedding of *E. coli* O157:H7 within a sheep model (305). Other researchers have begun using bacterial ghosts (e.g., cellular membranes) to reduce *E. coli* O157:H7 populations in mice (57); as well as using an attenuated *Salmonella* strain that expresses *E. coli* O157:H7 intimin proteins to induce immune responses in cattle (166). Others have devised chimeric multi-protein (eae, tir, intimin) vaccines (7) that can be produced in plants, potentially providing an edible vaccine (6) that can be included in cattle rations, an alternative to being injected, thus lowering expense to producers by reducing stressful handling procedures.

### Cattle Hide Washing

Currently, cattle hides are typically washed to remove visible contamination. Hide washes can contain antimicrobial compounds (e.g., organic acids [described above], sodium hydroxide, trisodium phosphate [TSP], cetylpyridinium chloride [CPC] or electrolyzed or ozonated water), which serve to reduce some of the bacterial contamination (including foodborne pathogens) entering the processing plant on the hide (17, 41-43). The most common hide/carcase rinse additives are organic acids such as lactic or acetic acid (35, 189). Hide washes significantly reduce the load of *E. coli* O157:H7 entering the plant on the hide, which has been linked to final carcase contamination levels (15, 19), thus improving food safety. However, they do not reduce the prevalence of *E. coli* O157:H7 entering the plant within the animal.

### Sodium Chlorate

Addition of chlorate to *E. coli* cultures kills bacteria. *E. coli* can respire under anaerobic conditions by reducing

nitrate to nitrite via the dissimilatory nitrate reductase enzyme (273). 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 (272). Chlorate treatment *in vitro* quickly reduced populations of *E. coli* O157:H7 and *Salmonella* (9). The addition of chlorate to animal rations reduced experimentally inoculated *E. coli* O157:H7 populations in swine and sheep intestinal tracts (12, 101) as well as *Salmonella* in intestinal contents (56). Other studies indicated soluble chlorate administered via drinking water significantly reduced *E. coli* O157:H7 in ruminal, cecal and fecal populations both in cattle and sheep (11, 61, 67). Hide contamination with *E. coli* O157:H7 plays a significant role in carcase/product contamination (18-20), and chlorate treatment reduces both fecal and hide populations of *E. coli* (13). *In vitro* and *in vivo* results indicated chlorate treatment does not adversely affect the ruminal or cecal/colonic fermentation (10). Additional studies demonstrate chlorate alters neither the antibiotic resistance nor toxin production by *E. coli* O157:H7 (58, 60). The lethal dose (LD50) of sodium chlorate is from 1.2 to 4 g/kg BW; by way of comparison, the LD50 of sodium chloride is approximately 3 g/kg BW (117). Therefore, it does not appear chlorate poses a severe risk for use in animals due to inherent toxicity.

Because of the dramatic impact chlorate has on foodborne pathogenic bacterial populations, it was suggested chlorate could be supplemented in the last feeding before cattle are shipped to the slaughterhouse. The use of chlorate to reduce foodborne pathogenic bacteria in food animals is presently under review by the U. S. Food and Drug Administration, but has not been approved at this time.

### WHAT ARE POTENTIAL UNINTENDED CONSEQUENCES?

Before we attempt to completely eliminate EHEC from the live animal, we must consider the law of unintended consequences, and its impact on food safety (66). The poultry industry was hampered in the early part of the 20th century by fowl typhoid/cholera which impacted productivity and efficiency of production. This disease was caused by *Salmonella* Gallinarum and Pullorum, which do not cause illness in humans, only in poultry (76). A concerted effort was made to rid the national poultry flock of these bacterial diseases, and this effort successfully eliminated these diseases which were highly adapted to live only in their host (poultry). However, by removing a member of the microbial ecosystem from the intestinal meta-population, a niche in the ecosystem was opened (169). This niche was occupied by another *Salmonella* that

was not host-adapted and was transmitted from rodents to poultry, *Salmonella* Enteritidis (169). This foodborne pathogen has become widespread in national poultry flocks and represents one of the most common serotypes isolated from human salmonellosis cases (76, 248). Therefore, in all our efforts to eliminate EHEC from animals prior to slaughter, we must be aware that some other bacteria will fill the vacancy in the ecosystem.

## CONCLUSION

Pre-harvest interventions to reduce *E. coli* O157:H7 and other EHEC in cattle hold potential to reduce foodborne pathogen dissemination on farms, in the environment, and entering the food chain. However, the development of these pre-harvest strategies does not eliminate the need for good sanitation and procedures in the processing plant and food preparation environment. Instead, live-animal interventions to reduce pathogens must be installed in a multiple-hurdle approach that complements in-plant interventions, so reduction in pathogen entry to the food supply can be maximized. Recent years have seen an increase in research into developing new interventions (e.g., vaccination, DFM, chlorate, phages) and into understanding what effect diet and the microbial population has on EHEC populations in the gut of cattle. This research has resulted in several novel interventions and potential dietary additions or changes that can reduce EHEC in cattle, including those already in, or very near to entering, the marketplace.

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