REVIEW
Current and near-market intervention strategies for reducing Shiga Toxin-Producing *Escherichia coli* (STEC) shedding in cattle

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ABSTRACT

Cattle can naturally contain foodborne pathogenic bacteria such as Shiga Toxin-Producing *E. coli* (STEC). These foodborne pathogenic bacteria are a threat to public health through contamination of foods and water supplies. In order to reduce human exposures and resultant illnesses, research has focused in recent years on the development of live animal intervention strategies that can be applied to reduce the burden of STEC entering the food chain. This review addresses the application of interventions that have been proposed or implemented to reduce STEC in live cattle. Recent years have seen increasing development of new interventions (e.g., vaccination, DFM, chlorate, phages) and into understanding what effect diet and the microbial population have on the microbial populations of the gut of cattle. This research has resulted in several novel interventions and potential dietary additions or changes that can reduce STEC in cattle, and many of them are in, or very near to entering, the marketplace. The live animal interventions must be designed in a coherent, complementary context as part of a multiple-hurdle scheme to reduce pathogens entry into the food supply.

Keywords: *Escherichia coli,* shiga toxin, intervention, cattle, shedding, near-market, multiple hurdle

INTRODUCTION

The beef industry has been significantly impacted by the emergence of Shiga toxin-producing *Esch-erichia coli* (STEC) bacteria which are naturally found in cattle (Karmali *et al.*, 2010). STEC-caused illnesses are a zoonotic disease (Karesh *et al.*, 2012) that costs the American economy more than $1 billion each year in direct and indirect costs from more than 175,000 human illnesses (Scallan *et al.*, 2011; Scharff, 2010). While strategies focused on the prevention of transmission via carcasses have been largely suc-

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cessful, they are far from perfect (Arthur *et al.*, 2007a; Barkocy-Gallagher *et al.*, 2003). Thus it has been necessary to develop animal management controls as well as applicable intervention strategies for use in live cattle (Callaway *et al.*, 2004b; LeJeune and Wetzel, 2007; Oliver *et al.*, 2008; Sargeant *et al.*, 2007).

Because human STEC exposures are not limited only to food-based routes, but include animal contact, it is likely that reducing STEC in cattle can improve public health in rural communities, as well as in reducing foodborne illnesses (LeJeune and Kersting, 2010; Rotariu *et al.*, 2012). As discussed previously (Callaway *et al.*, 2013) the logic underlying focusing on reducing foodborne pathogenic bacteria in live cattle is straightforward: 1) reducing the amount of pathogens entering processing plants will reduce the burden on the plants and render the in-plant interventions more effective; 2) reducing horizontal pathogen spread from infected animals (especially in "supershedders") in transport and lairage; 3) will reduce the pathogenic bacterial burden in the environment and wastewater streams; and 4) will reduce the direct risk to those in direct contact with animals via petting zoos, open farms, rodeos and to animal workers.

This present review is intended to complement the accompanying STEC ecology and animal management-focused review (Callaway *et al.*, 2013) and will stress the application of external intervention strategies focused on reducing STEC in live cattle. We will divide the interventions into two broad categories: 1) Probiotic approaches that utilize the competitive nature of the gastrointestinal microbiome, and 2) Anti-pathogen strategies that specifically target pathogens based on their physiology and ecological niches.

PROBIOTIC APPROACHES, HARNESSING MICROBIAL ECOLOGY

In recent years, probiotic approaches (e.g., those that utilize live or dead cultures of microorganisms to alter the microbial population of the gut) have received increased interest as a method to reduce

foodborne pathogenic bacteria in cattle. Traditionally, probiotic products in the cattle industry have been used to enhance production efficiency of meat or milk (Callaway and Martin, 2006; Fuller, 1989; Tournut, 1989; Yoon and Stern, 1996). However recent years have an increase in the use of the probiotic types: direct fed microbials (DFM), competitive exclusion cultures (CE), and prebiotics to reduce *E. coli* O157:H7 populations in cattle (McAllister *et al.*, 2011) and can be considered part of an "organic" approach to improving food safety (Siragusa and Ricke, 2012).

In general it appears that probiotic products work to 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 (Collins and Gibson, 1999; Kim *et al.*, 2008). Volatile fatty acids produced by microbial fermentation can be toxic to some bacterial species (Ricke, 2003; Russell, 1992; Wolin, 1969), and 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 (Jack *et al.*, 1995). Collectively, these modes of action demonstrate the complexities involved with interrupting the cycle of transmission and colonization of cattle with *E. coli* O157:H7, and emphasize that a multiple-hurdle using complementary interventions has the greatest chance of improving food safety at the live animal level.

Direct Fed Microbials

Direct Fed Microbials are widely fed in 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. A DFM is fed to animals daily to improve the ruminal fermentation and production efficiency (Martin and Nisbet, 1992). Increasingly, companies claim some benefit to them in reducing *E. coli* O157:H7 shed-

ding in cattle. Researchers compared several of the commercially-available growth enhancement probiotics and yeast products and found that feeding these probiotics provided no effect in regards to pathogen levels in cattle (Keen and Elder., 2000; Swyers et al., 2011). A probiotic culture comprised of *Streptococcus bovis* and *Lactobacillus gallinarum* from the rumen of cattle reduced *E. coli* O157 shedding when given to experimentally-infected calves, and this decrease was attributed to an increase in VFA concentration in the gut (Ohya et al., 2001). Probiotic products have been developed to specifically reduce *E. coli* O157:H7 shedding in cattle. A probiotic that contained 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 in sheep, yet, a monoculture of *Lactobacillus acidophilus* was found to be ineffective (Lema et al., 2001). A DFM comprised of *Bacillus subtilis* did not affect the fecal prevalence or concentration of *E. coli* O157:H7 and did not impact average daily gain in feedlot cattle (Arthur et al., 2010a). Studies have also indicated that cultures of *Lactobacillus acidilacti* and *Pediococus* could directly inhibit *E. coli* O157:H7, likely through the production of organic acids and low pH (Rodriguez-Palacios et al., 2009).

Other researchers demonstrated that a direct-fed-microbial (DFM) *L. acidophilus* culture derived directly from the rumen of cattle reduced *E. coli* O157:H7 shedding by more than 50% when fed to feedlot cattle (Brashears and Galyean, 2002; Brashears et al., 2003a; Brashears et al., 2003b). In an independent evaluation, this DFM reduced fecal shedding of *E. coli* O157:H7 in cattle from 46% to 13% (Ransom et al., 2003). In a further refinement of this DFM, where the *L. acidophilus* cultures were combined with *Propionibacterium freudenreichii* (a propionate-producing commensal intestinal bacteria) a reduction in the prevalence of *E. coli* O157:H7 occurred in the feces from approximately 27% to 16% and reduced the prevalence on hides from 14% to 4% (Elam et al., 2003; Younts-Dahl et al., 2004). Further work with this DFM again showed that it reduced *E. coli* O157:H7 and *Salmonella* in feces and on hides (Stephens et al., 2007b), and it further reduced concentrations of *E. coli* O157:H7 in the feces (Stephens et al., 2007a; Stephens et al., 2007b), which may be more of a critical impactor of carcass contamination than simple prevalence levels (Arthur et al., 2010b). Additional studies using only the *L. acidophilus* DFM found no impact of low dose DFM feeding on *E. coli* O157:H7 prevalence (Cull et al., 2012). It is important to note that in this study a low dose DFM product was utilized, and further research indicates that the effect on *E. coli* O157:H7 prevalence and concentrations is impacted by DFM dosage levels (Cull et al., 2012).

This *Lactobacillus*-based DFM is currently marketed as Bovamine™ and Bovamine Defend™ based on dosing levels and both are widely used in the cattle industry because they have been reported to improve the growth efficiency of cattle, at least in a feedlot ration. There will likely not be a single DFM that can work effectively at reducing *E. coli* O157:H7 populations in cattle and improve production efficiency in all production systems (i.e., feedlots, cow-calf, stockers, and dairies). Therefore, alternative DFM cultures selected specifically for each production segment or situation need to be developed so that the 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

Competitive exclusion (CE) is another probiotic approach that has been used to eliminate *E. coli* O157:H7 (as well as *Salmonella*) from cattle gastrointestinal tracts (Brashears and Galyean, 2002; Brashears et al., 2003a; Brashears et al., 2003b; Zhao et al., 2003). 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 (Fuller, 1989; Nurmi et al., 1992). An established gastrointestinal microbial population makes an animal more resistant to transient opportunistic infections (Fuller, 1989), because the species

best adapted to occupy a particular niche within the intestinal tract succeeds, and 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 (Nurmi et al., 1992). 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 (Fox et al., 2009a; Reissbrodt et al., 2009; Zhao et al., 1998). Researchers used a defined population of multiple commensal E. coli strains that were isolated from cattle and found this generic E. coli CE culture could displace an established E. coli O157:H7 population from calves (Zhao et al., 1998). In a follow up study, calves that were colonized with the E. coli CE product shed less E. coli O111:NM and O26:H111 (both STEC strains isolated from cattle, but the CE product did not reduce E. coli O157:H7 (Zhao et al., 2003). Other researchers have isolated E. coli strains that display a "proximity-dependent" killing of E. coli O157:H7 strains which could possibly be utilized in CE cultures or as a DFM (Sawant et al., 2011). While the mechanism of this killing has not been defined, it does not appear to be mediated by colicins or phages (Sawant et al., 2011).

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 (Konisky, 1982; Lakey and Slatin, 2001; Smarda and Smajs, 1998), including E. coli O157:H7 (Jordi et al., 2001; Murinda et al., 1996; Schamberger and Diez-Gonzalez, 2002). The concept of using colicins as an intervention strategy to kill food borne pathogens is not new (Joerger, 2003; Murinda et al., 1996), but until recently has been lim-

ited by cost to use as treatment on finished meat products (Abercrombie et al., 2006; Liu et al., 2011; Patton et al., 2008) or vegetables (Nandiwada et al., 2004). Recently however, the costs of production and purification of colicins was lowered by recombination protein expression work (Stahl et al., 2004). Because of the increased availability of the colicins, scaled up studies could be conducted in a mouse model, where it was demonstrated that E. coli O157:H7 was prevented from colonization (Leatham et al., 2009). Recently, specific studies have examined the use of specific colicins against E. coli O157:H7 in vitro in gastrointestinal simulations (Callaway et al., 2004d) and against other E. coli in vivo (Cutler et al., 2007).

In spite of the seemingly simple addition of a protein (colicin) to animal diets to control E. coli O157:H7, studies have indicated that the sensitivity of E. coli O157:H7 strains to any single colicin can be highly variable (Murinda et al., 1996; Murinda et al., 1998; Schamberger and Diez-Gonzalez, 2002). Because some E. coli O157:H7 strains are colicinogenic and produce specific concomitant immunity proteins (Murinda et al., 1998), these strains of E. coli O157:H7 can be resistant to certain added colicins or even a broad category of colicins (Alonso et al., 2000). Therefore, if colicins are to be used as a preharvest intervention strategy, there must be simultaneous administration of several categories of colicins. 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. As a way of getting colicins into the lower gut of cattle, researchers have proposed a specific form of DFM/CE of feeding colicin-producing E. coli in cattle rations (Schamberger and Diez-Gonzalez, 2002; Schamberger et al., 2004; Zhao et al., 1998). These strains have been shown to colonize the lower gut of cattle, but the reduction in concentration of E. coli O157 was approximately 2 log10 CFU/g, not a complete elimination (Nandiwada et al., 2004).

The complex nature of ruminant animal gastrointestinal tract, and the long (12-18 month) life span of cattle going into a feedlot means that CE use in cattle as a "one shot" approach may not completely eliminate E. coli O157:H7 and other STEC 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 an early-established CE culture may be best supplemented over time by DFM and/or prebiotic feeding (synbiotics, described below).

Prebiotics

Organic compounds that are unavailable to, or indigestible by the host animal, but are digestible by a specific segment of the microbial population are generally classified as "prebiotics" (Patterson and Burkholder, 2003; Schrezenmeir and De Vrese, 2001; Walker and Duffy, 1998). For example, fructo-oligo-saccharides, are sugars that are 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 (Houdijk *et al.*, 1998; Willard *et al.*, 2000). Some prebiotics can provide a competitive advantage to specific members of the native microflora (e.g., *Bifidobacteria, Butyrivibrio*) that can help to exclude pathogenic bacteria from the intestine via direct competition for nutrients or for binding sites through the production of "blocking factors", or antimicrobial compounds in a fashion similar to that of CE (Zopf and Roth, 1996). Other prebiotics (Celmanax) have been shown to have an anti-adhesive effect on *E. coli* O157:H7 *in vitro* using bovine cells, which should be investigated further (Baines *et al.*, 2011).

Coupling the use of CE and prebiotics is known as "synbiotics", and could yield a synergistic effect in reduction of food-borne pathogenic bacterial populations in food animals prior to slaughter (Bomba *et al.*, 2002). 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.

ANTI-PATHOGEN STRATEGIES, TARGETED TREATMENT

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

Antibiotics

The use of antibiotics specifically to control *E. coli* O157:H7 shedding in cattle is controversial. Few researchers have delved into this area in cattle to date. Neomycin is an antibiotic that is approved for use in cattle to treat enteric infections and has been shown to reduce *E. coli* O157:H7 populations in the gut (Elder *et al.*, 2002; Ransom *et al.*, 2003) and on the hides of cattle (Ransom *et al.*, 2003). Other researchers have found that in swine artificially infected with *E. coli* O157:H7, the feeding of chlortetracycline and tylosin decreased fecal shedding, while bacitracin did not impact *E. coli* O157:H7 populations (Cornick, 2010). It is hypothesized that the generalized disruption of the microbial ecosystem that is caused by antibiotic treatment indirectly affects the *E. coli* O157:H7 populations; the use of some antibiotics thus 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.

Bacteriophages

Bacteria can be infected by naturally-occurring bacteriophages (bacterial viruses) that are found in many environments (Kutter and Sulakvelidze, 2005; Lederberg, 1996), including the intestinal tract of cattle (Callaway *et al.*, 2006; Goodridge, 2008; Go-

odridge, 2010). 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 (Lederberg, 1996). This specificity should allow phages to be used as an anti-pathogen treatment, a kind of "smart bomb" targeting on the species we wish to eliminate, without perturbing the overall microbial ecosystem (Johnson *et al.*, 2008). Lytic 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 (Tanji *et al.*, 2005).

Phages have been examined for use in two different approaches to reduce *E. coli* O157:H7, within the gut of cattle before slaughter, and as a hide or environmental decontaminant (Ricke *et al.*, 2012). Commercial phage-based anti-*E. coli* O157:H7 are currently 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 marketplaces (Omnilytics and Elanco, Finalyse). Company-based research indicates a significant reduction in positive trim samples from cattle that were sprayed with this product. Processors are finding appropriate critical control points in which to include phage sprays on carcasses prior to de-hiding 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. Several phages isolated by European laboratories have shown promise as *E. coli* O157:H7 reduction agents sprayed on cattle hides,

but that they require an extended exposure time (1 h) to obtain maximal effect (Coffey *et al.*, 2011). Interestingly, several phages have been isolated recently that are effective both against *Salmonella* spp. and *E. coli* O157:H7 (López-Cuevas *et al.*, 2011; López-Cuevas *et al.*, 2012; Park *et al.*, 2012), which offers the hope of phage use as a broad-spectrum food safety improvement.

Phages have been used successfully in several *in vivo* research studies examining the effect of phage on diseases that impact animal production efficiency or health (Huff *et al.*, 2002; Smith and Huggins, 1982; 1983; 1987). Bacteriophage treatment reduced enterotoxigenic *E. coli* (ETEC)-induced diarrhea and splenic ETEC colonization in calves (Smith and Huggins, 1983; 1987). With the increasing focus on improving food safety throughout the food production continuum, bacteriophages have been used to control experimentally inoculated foodborne pathogenic bacteria, especially *E. coli* O157:H7 in cattle gastrointestinal tracts (Bach *et al.*, 2003; Bach *et al.*, 2009; Callaway *et al.*, 2008; Kudva *et al.*, 1999; Niu *et al.*, 2008; Rozema *et al.*, 2009). Several different phages have been isolated from feedlot cattle (Callaway *et al.*, 2006; Niu *et al.*, 2009; Niu *et al.*, 2012; Oot *et al.*, 2007) and other sources (Liu *et al.*, 2012; McLaughlin *et al.*, 2006) and have been used to reduce *E. coli* O157:H7 strains in experimentally-infected animals as proofs of concept (Bach *et al.*, 2009; Callaway *et al.*, 2008; Rivas *et al.*, 2010). In other studies, naturally phage-infected ruminants have been shown to be more resistant to *E. coli* O157:H7 colonization (Raya *et al.*, 2006) and the presence of these endemic phages have often confused results of intervention studies (Kropinski *et al.*, 2012). Commercialization studies for these on farm products have had mixed results (Stanford *et al.*, 2010), but studies focusing on the development of appropriate, effective multi-phage cocktails are currently underway (Stanford and McAllister, personal communication). No matter what point in the beef production chain the phages are utilized in (e.g., hides or in the live animal), they must be carefully selected for: 1) action against multiple *E. coli* O157:H7 strains as well as other non-O157 STEC strains, 2) members

of a cocktail must utilize different receptors to minimize resistance development, and 3) must be strictly lytic (i.e., does not transfer genetic material) because phage-mediated transfer is the mechanism by which STEC originally acquired their Shiga-toxin genes (Brabban et al., 2005; Law, 2000).

Vaccination

Immunization has worked very effectively against pathogenic bacteria, including E. coli strains that cause edema disease in pigs and Salmonella in poultry (Gyles, 1998; Johansen et al., 2000). Unfortunately, because EHEC/STEC do not cause disease in cattle, the immunostimulation provided by these foodborne pathogens is not as potent, because it appears that natural exposure to E. coli O157:H7 does not confer protection to the host (Gyles, 1998). Thus vaccine production has specifically targeted aspects of the physiology of E. coli O157:H7 (Walle et al., 2012). 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. To date, two basic targeting strategies have been utilized to develop vaccines against E. coli O157:H7, and both have had their successes (Snedeker et al., 2012; Varela et al., 2013; Walle et al., 2012).

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 utilizes secreted 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 EpitopixTM SRP vaccine has been conditionally approved for use in cattle in the U.S. and is undergoing additional safety and efficacy tests. Preliminary research results are promising when the vaccine is utilized in a 3 dose treatment regimen (Thornton et al., 2009). Other researchers found that vaccination with the SRP reduced fecal concentrations of E. coli O157:H7 in cattle by 98%, but the vaccine did not affect cattle performance (Thomson et al., 2009). Vaccination of cattle with this SRP in another study reduced the prevalence of E. coli O157:H7 by nearly 50% (Fox et al., 2009b). A two-dose SRP vaccination reduced the prevalence and number of "high-shedding" cattle, with a reported efficacy of 53% and 77%, respectively (Cull et al., 2012). Vaccination of pregnant dams along with a second vaccination of calves was shown to reduce E. coli O157:H7 (from 25% to 15%, respectively) in feedlot cattle (Wileman et al., 2011).

Bacterial Extract Vaccines

A vaccine produced from E. coli O157:H7 extracts (type III secreted proteins) has been produced as EconicheTM. This vaccine has been licensed in Canada and is pending a conditional license in the U.S. Preliminary experimental results indicated that this vaccine reduced E. coli O157:H7 shedding in feedlot cattle from 23% to less than 9% (Moxley et al., 2003; Potter et al., 2004; Van Donkersgoed et al., 2005). In an evaluation study, it was demonstrated that vaccination reduced fecal shedding from 46% to 14% (Ransom et al., 2003). Recent studies have shown an experimental three dose regimen reduced E. coli O157:H7 shedding by 65%, but that a 2 dose system was less effective (Moxley et al., 2009). However, in a follow up study, a two dose regimen was shown to reduce rectal colonization by E. coli O157:H7 in feedlot cattle (Smith et al., 2009b). The benefits of vaccinating cattle in reducing cattle hides positive for E. coli O157:H7 can be lost by comingling with non-vaccinated cattle during transport (Smith et al., 2009a).

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 that reduce fecal shedding in experimental-infection models (McNeilly et al., 2010); vaccines against a hemolysin pro-

tein encoded in the locus of enterocyte effacement (LEE) island has also shown promise in reducing *E. coli* O157:H7 shedding in cattle (Sharma *et al.*, 2011). Vaccines targeting EspA, EspB, shiga-toxin 2, and Intimin proteins have been used in pregnant cows, and it was shown that the antibodies were transferred to calves, but the effect of this vaccination on colonization was not determined (Rabinovitz *et al.*, 2012). Further multi-protein vaccines have been developed that can reduce fecal shedding of *E. coli* O157:H7 in a sheep model (Yekta *et al.*, 2011), including a Stx2B-Tir-Stx1B-Zot protein vaccine that successfully reduced *E. coli* O157:H7 shedding in a goat model (Zhang *et al.*, 2012). Most excitingly, because the non-O157 STEC share the Type-III secretion system proteins, it appears that vaccines targeting these proteins (e.g., Tir, EspB, EspD, EspA, and NleA) can provide some degree of cross-protection from the non-O157 STEC (Asper *et al.*, 2011).

Bacterial ghosts (e.g., cellular membranes) have recently been used to produce an immune response that reduced *E. coli* O157:H7 populations in mice (Cai *et al.*, 2010; Mayr *et al.*, 2012) and calves (Vilte *et al.*, 2012). A live-attenuated *Salmonella* strain that expresses the *E. coli* O157:H7 intimin protein has been demonstrated to induce immune responses in cattle (Khare *et al.*, 2010). Others have devised chimeric multi-protein (eae, tir, intimin) vaccines (Amani *et al.*, 2010) that can be produced in plants, potentially providing a source of an edible vaccine (Amani *et al.*, 2011) that can be included in cattle rations rather than having to be injected via the stressful handling procedures currently required that add expense to the producers. However, for this approach to be utilized in ruminants, the proteins must be protected from the extensive proteolytic nature of the rumen microbial ecosystem, which will obviously add to the complexity and expense of vaccination via the edible vaccine approach.

Cattle Hide washing

Currently, cattle hides are typically washed to remove visible contamination from hides. The hide washes can contain antimicrobial compounds (e.g., organic acids [described in previous section], sodium hydroxide, trisodium phosphate [TSP], cetylpyridinium chloride [CPC] , hypobromous acid, or electrolyzed or ozonated water), which serves to reduce some of the bacterial contamination (including foodborne pathogens) entering the processing plant on the hide (Arthur *et al.*, 2007b; Bosilevac *et al.*, 2004; Bosilevac *et al.*, 2005a; Bosilevac *et al.*, 2005b; Schmidt *et al.*, 2012). The most common hide/carcass rinse additive has been organic acids such as lactic or acetic acid (Berry and Cutter, 2000; Loretz *et al.*, 2011). Hide washes significantly reduce the load of *E. coli* O157:H7 entering the plant on the hide, which has been linked to final carcass contamination levels (Arthur *et al.*, 2007a; Arthur *et al.*, 2010b), thus improving food safety; but 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 these bacteria because *E. coli* can respire under anaerobic conditions by reducing nitrate to nitrite via the dissimilatory nitrate reductase enzyme (Stouthamer, 1969). The intracellular bacterial enzyme nitrate reductase does not differentiate between nitrate and its analog, chlorate which is reduced to chlorite in the cytoplasm; chlorite accumulation kills bacteria (Stewart, 1988). Chlorate treatment *in vitro* quickly reduced populations of *E. coli* O157:H7 and *Salmonella* (Anderson *et al.*, 2000a). Chlorate addition to animal rations reduced experimentally inoculated *E. coli* O157:H7 populations in swine and sheep intestinal tracts (Anderson *et al.*, 2001; Edrington *et al.*, 2003) as well as *Salmonella* in broiler intestinal contents (Byrd *et al.*, 2003). Other studies indicated that soluble chlorate administered via drinking water significantly reduced *E. coli* O157:H7 ruminal, cecal and fecal populations in both cattle and sheep (Anderson *et al.*, 2002; Callaway *et al.*, 2002; Callaway *et al.*, 2003). Hide contamination with *E. coli* O157:H7 plays a significant role in carcass/product contami-

nation (Arthur *et al.*, 2009; Arthur *et al.*, 2010a; Arthur *et al.*, 2010b), and chlorate treatment reduces both fecal and hide populations of *E. coli* (Anderson et al., 2005). *In vitro* and *in vivo* results have indicated that chlorate treatment does not adversely affect the ruminal or the cecal/colonic fermentation (Anderson *et al.*, 2000b). Additional studies have demonstrated that chlorate alters neither the antibiotic resistance, nor toxin production by *E. coli* O157:H7 (Callaway *et al.*, 2004a; Callaway *et al.*, 2004c). The LD_{50} of sodium chlorate is from 1.2 to 4 g/kg BW; by way of comparison, the LD_{50} of sodium chloride is approximately 3 g/kg BW (Fiume, 1995). Therefore, it does not appear that chlorate poses a severe risk for use in animals due to inherent toxicity.

Because of the dramatic impact chlorate has on food-borne pathogenic bacterial populations, it was suggested that chlorate could be supplemented in the last feeding before cattle are shipped to the slaughterhouse. The use of chlorate to reduce food-borne 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 ABOUT POTENTIAL UNINTENDED CONSEQUENCES?

Before we attempt to completely eliminate STEC from the live animal, we must consider the law of unintended consequences, and its impact on food safety (Callaway *et al.*, 2007). 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, but do cause illness solely in poultry (CDC, 2006). A concerted effort was made to rid the national poultry flock of these bacterial diseases, and this effort was successful at eliminating 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 (Kingsley and Bäumler, 2000).

This niche was occupied by another *Salmonella* that was not host-adapted and was transmitted from rodents to poultry, *Salmonella* Enteritidis (Kingsley and Bäumler, 2000). This foodborne pathogen has subsequently become widespread in the national poultry flocks and represents one of the most common serotypes isolated from human salmonellosis cases (CDC, 2006; Scallan *et al.*, 2011). Therefore, in all our efforts to eliminate STEC from animals prior to slaughter, we must be aware that some other bacteria will undoubtedly fill the vacuum in the microbial ecosystem.

CONCLUSIONS

Pre-harvest interventions to reduce *E. coli* O157:H7 and other STEC in cattle can reduce foodborne pathogen penetration into the food chain. However, implementation of these pre-harvest strategies does not eliminate the need for best practices in the processing plant and in the food preparation environment. Recent years have seen an increase in the research into developing new interventions (e.g., vaccination, DFM, chlorate, phages) and into understanding what effect the microbial population and host physiology has on STEC populations in the gut of cattle. This research has resulted in several novel interventions and potential dietary additions or changes that can reduce STEC in cattle, and many of them are in, or very near to entering, the marketplace. However, it must be noted that the live-animal interventions must be installed in a coherent, complementary fashion to reduce pathogens as part of an integrated multiple-hurdle approach that complements other post-harvest strategies to minimize pathogen contact and resultant human illnesses.

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