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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

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INTRODUCTION

The beef industry has been significantly impacted by the emergence of Shiga toxin-producing *Escherichia 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 limited 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-oligosaccharides, 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 Econiche™. 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 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 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.

REFERENCES

Abercrombie, J. G., M. J. B. Paynter and S. S. Hayasaka. 2006. Ability of colicin V to control *Escherichia coli* O157:H7 in ground beef. J. Food Safety 26:103-114.

Alonso, G., G. Vilchez and R. L. Vidal. 2000. How bacteria protect themselves against channel-forming colicins. Inter. Microbiol. 3:81-88.

Amani, J., S. L. Mousavi, S. Rafati and A. H. Salmanian. 2011. Immunogenicity of a plant-derived edible chimeric *EspA*, *Intimin* and *Tir* of *Escherichia coli* O157:H7 in mice. Plant Sci. 180:620-627.

Amani, J., A. H. Salmanian, S. Rafati and S. L. Mousavi. 2010. Immunogenic properties of chimeric protein from *espA*, *eae* and *tir* genes of *Escherichia coli* O157:H7. Vaccine. 28:6923-6929.

Anderson, R. C., S. A. Buckley, L. F. Kubena, L. H. Stanker, R. B. Harvey and D. J. Nisbet. 2000a. Bactericidal effect of sodium chlorate on *Escherichia coli* O157:H7 and *Salmonella* Typhimurium DT104 in rumen contents *in vitro*. J. Food Prot. 63:1038-1042.

Anderson, R. C., S. A. Buckley, L. H. Stanker, L. F. Kubena, R. B. Harvey and D. J. Nisbet. 2000b. Effect of sodium chlorate on *Escherichia coli* concentration in the bovine gut. Microb. Ecol. Health Dis. 12:109-116.

Anderson, R. C., T. R. Callaway, T. J. Anderson, L. F. Kubena, N. K. Keith and D. J. Nisbet. 2002. Bactericidal effect of sodium chlorate on *Escherichia coli* concentrations in bovine ruminal and fecal contents *in vivo*. Microb. Ecol. Health Dis. 14:24-29.

Anderson, R. C., T. R. Callaway, S. A. Buckley, T. J. Anderson, K. J. Genovese, C. L. Sheffield and D. J. Nisbet. 2001. Effect of oral sodium chlorate administration on *Escherichia coli* O157:H7 in the gut of experimentally infected pigs. Int. J. Food Microbiol. 71:125-130.

Anderson, R. C., M. A. Carr, R. K. Miller, D. A. King, G. E. Carstens, K. J. Genovese, T. R. Callaway, T. S. Edrington, Y. S. Jung, J. L. McReynolds, M. E. Hume, R. C. Beier, R. O. Elder and D. J. Nisbet. 2005. Effects of experimental chlorate preparations as feed and water supplements on *Escherichia coli* colonization and contamination of beef cattle and carcasses. Food Microbiol. 22:439-447.

Arthur, T. M., J. M. Bosilevac, D. M. Brichta-Harhay, M. N. Guerini, N. Kalchayanand, S. D. Shackelford, T. L. Wheeler and M. Koohmaraie. 2007a. Transportation and lairage environment effects on prevalence, numbers, and diversity of *Escherichia coli* O157:H7 on hides and carcasses of beef cattle at processing. J. Food Prot. 70:280-286.

Arthur, T. M., J. M. Bosilevac, D. M. Brichta-Harhay, N. Kalchayanand, S. D. Shackelford, T. L. Wheeler and M. Koohmaraie. 2007b. Effects of a minimal hide wash cabinet on the levels and prevalence of *Escherichia coli* O157:H7 and *Salmonella* on the hides of beef cattle at slaughter. J. Food Prot. 70:1076-1079.

Arthur, T. M., J. M. Bosilevac, N. Kalchayanand, J. E. Wells, S. D. Shackelford, T. L. Wheeler and M. Koohmaraieie. 2010a. Evaluation of a direct-fed microbial product effect on the prevalence and load of *Escherichia coli* O157:H7 in feedlot cattle. J. Food Prot. 73:366-371.

Arthur, T. M., D. M. Brichta-Harhay, J. M. Bosilevac, N. Kalchayanand, S. D. Shackelford, T. L. Wheeler and M. Koohmaraie. 2010b. Super shedding of *Escherichia coli* O157:H7 by cattle and the impact on beef carcass contamination. Meat Sci. 86:32-37.

Arthur, T. M., J. E. Keen, J. M. Bosilevac, D. M. Brichta-Harhay, N. Kalchayanand, S. D. Shackelford, T. L. Wheeler, X. Nou and M. Koohmaraie. 2009. Longitudinal study of *Escherichia coli* O157:H7 in a beef cattle feedlot and role of high-level shedders in hide contamination. Appl. Environ. Microbiol. 75:6515-6523.

Asper, D. J., M. A. Karmali, H. Townsend, D. Rogan and A. A. Potter. 2011. Serological response of shiga toxin-producing *Escherichia coli* type III secreted proteins in sera from vaccinated rabbits, naturally infected cattle, and humans. Clin. Vacc. Immunol. 18:1052-1057.

Bach, S. J., R. P. Johnson, K. Stanford and T. A. McAllister. 2009. Bacteriophages reduce *Escherichia coli* O157:H7 levels in experimentally inoculated sheep. Can. J. Anim. Sci. 89:285-293.

Bach, S. J., T. A. McAllister, D. M. Veira, V. P. J. Gannon and R. A. Holley. 2003. Effect of bacteriophage DC22 on *Escherichia coli* O157:H7 in an artificial rumen system (RUSITEC) and inoculated sheep. Anim. Res. 52:89-101.

Baines, D., S. Erb, R. Lowe, K. Turkington, E. Sabau,

G. Kuldau, J. Juba, L. Masson, A. Mazza and R. Roberts. 2011. A prebiotic, Celmanax, decreases *Escherichia coli* O157:H7 colonization of bovine cells and feed-associated cytotoxicity *in vitro*. BMC Res. Notes. 4:110-118.

Barkocy-Gallagher, G. A., T. M. Arthur, M. Rivera-Betancourt, X. Nou, S. D. Shackelford, T. L. Wheeler and M. Koohmaraie. 2003. Seasonal prevalence of shiga toxin-producing *Escherichia coli*, including O157:H7 and non-O157 serotypes, and *Salmonella* in commercial beef processing plants. J. Food Prot. 66:1978-1986.

Berry, E. D. and C. N. Cutter. 2000. Effects of acid adaptation of *Escherichia coli* O157:H7 on efficacy of acetic acid spray washes to decontaminate beef carcass tissue. Appl. Environ. Microbiol. 66:1493-1498.

Bomba, A., R. Nemcová, D. Mudronová and P. Guba. 2002. The possibilities of potentiating the efficacy of probiotics. Trends Food Sci. Technol. 13:121-126.

Bosilevac, J. M., T. M. Arthur, T. L. Wheeler, S. D. Shackelford, M. Rossman, J. O. Reagan and M. Koohmaraie. 2004. Prevalence of *Escherichia coli* O157 and levels of aerobic bacteria and enterobacteriaceae are reduced when hides are washed and treated with cetylpyridinium chloride at a commercial beef processing plant. J. Food Prot. 67:646-650.

Bosilevac, J. M., X. Nou, M. S. Osborn, D. M. Allen and M. Koohmaraie. 2005a. Development and evaluation of an on-line hide decontamination procedure for use in a commercial beef processing plant. J. Food Prot. 68:265-272.

Bosilevac, J. M., S. D. Shackelford, D. M. Brichta and M. Koohmaraie. 2005b. Efficacy of ozonated and electrolyzed oxidative waters to decontaminate hides of cattle before slaughter. J. Food Prot. 68:1393-1398.

Brabban, A. D., E. Hite and T. R. Callaway. 2005. Evolution of foodborne pathogens via temperate bacteriophage-mediated gene transfer. Foodborne Path. Dis. 2:287-303.

Brashears, M. M. and M. L. Galyean. 2002. Testing of probiotic bacteria for the elimination of *Escherichia coli* O157:H7 in cattle. http://foodsafety.ksu.edu/articles/263/probiotic_Ecoli_cattle.pdf. 26 April, Accessed 2013.

Brashears, M. M., M. L. Galyean, G. H. Loneragan, J. E. Mann and K. Killinger-Mann. 2003a. Prevalence of *Escherichia coli* O157:H7 and performance by beef feedlot cattle given *Lactobacillus* direct-fed microbials. J. Food Prot. 66:748-754.

Brashears, M. M., D. Jaroni and J. Trimble. 2003b. Isolation, selection, and characterization of lactic acid bacteria for a competitive exclusion product to reduce shedding of *Escherichia coli* O157:H7 in cattle. J. Food Prot. 66:355-363.

Byrd, J. A., R. C. Anderson, T. R. Callaway, R. W. Moore, K. D. Knape, L. F. Kubena, R. L. Ziprin and D. J. Nisbet. 2003. Effect of experimental chlorate product administration in the drinking water on *Salmonella* Typhimurium contamination of broilers. Poult. Sci. 82:1403-1406.

Cai, K., X. Gao, T. Li, X. Hou, Q. Wang, H. Liu, L. Xiao, W. Tu, Y. Liu, J. Shi and H. Wang. 2010. Intragastric immunization of mice with enterohemorrhagic *Escherichia coli* O157:H7 bacterial ghosts reduces mortality and shedding and induces a Th2-type dominated mixed immune response. Can. J. Microbiol. 56:389-398.

Callaway, T. R., R. C. Anderson, T. S. Edrington, K. M. Bischoff, K. J. Genovese, T. L. Poole, J. A. Byrd, R. B. Harvey and D. J. Nisbet. 2004a. Effects of sodium chlorate on antibiotic resistance in *Escherichia coli* O157:H7. Foodborne Path. Dis. 1:59-63.

Callaway, T. R., R. C. Anderson, T. S. Edrington, K. J. Genovese, R. B. Harvey, T. L. Poole and D. J. Nisbet. 2004b. Recent pre-harvest supplementation strategies to reduce carriage and shedding of zoonotic enteric bacterial pathogens in food animals. Anim. Health Res. Rev. 5:35-47.

Callaway, T. R., R. C. Anderson, T. S. Edrington, Y. S. Jung, K. M. Bischoff, K. J. Genovese, T. L. Poole, R. B. Harvey, J. A. Byrd and D. J. Nisbet. 2004c. Effects of sodium chlorate on toxin production by *Escherichia coli* O157:H7. Curr. Iss. Intest. Microbiol. 5:19-22.

Callaway, T. R., R. C. Anderson, K. J. Genovese, T. L. Poole, T. J. Anderson, J. A. Byrd, L. F. Kubena and

D. J. Nisbet. 2002. Sodium chlorate supplementation reduces *E. coli* O157:H7 populations in cattle. J. Anim. Sci. 80:1683-1689.

Callaway, T. R., T. S. Edrington, R. C. Anderson, J. A. Byrd and D. J. Nisbet. 2007. Gastrointestinal microbial ecology and the safety of our food supply as related to *Salmonella*. J. Anim. Sci. 86:167-172.

Callaway, T. R., T. S. Edrington, R. C. Anderson, K. J. Genovese, T. L. Poole, R. O. Elder, J. A. Byrd, K. M. Bischoff and D. J. Nisbet. 2003. *Escherichia coli* O157:H7 populations in sheep can be reduced by chlorate supplementation. J. Food Prot. 66:194-199.

Callaway, T. R., T. S. Edrington, A. D. Brabban, R. C. Anderson, M. L. Rossman, M. J. Engler, M. A. Carr, K. J. Genovese, J. E. Keen, M. L. Looper, E. M. Kutter and D. J. Nisbet. 2008. Bacteriophage isolated from feedlot cattle can reduce *Escherichia coli* O157:H7 populations in ruminant gastrointestinal tracts. Foodborne Path. Dis. 5:183-192.

Callaway, T. R., T. S. Edrington, A. D. Brabban, J. E. Keen, R. C. Anderson, M. L. Rossman, M. J. Engler, K. J. Genovese, B. L. Gwartney, J. O. Reagan, T. L. Poole, R. B. Harvey, E. M. Kutter and D. J. Nisbet. 2006. Fecal prevalence of *Escherichia coli* O157, *Salmonella*, *Listeria*, and bacteriophage infecting *E. coli* O157:H7 in feedlot cattle in the southern plains region of the United States. Foodborne Path. Dis. 3:234-244.

Callaway, T. R., T. S. Edrington, G. H. Loneragan, M. A. Carr and D. J. Nisbet. 2013. Shiga Toxin-producing *Escherichia coli* (STEC) ecology in cattle and management based options for reducing fecal shedding. Agric. Food Anal. Bacteriol. 3:39-69.

Callaway, T. R. and S. A. Martin. 2006. Use of fungi and organic acids in production animal diets. In: Ed. Feedstuffs Direct-fed Microbial, Enzyme and Forage Additive Compendium, 8th Ed. Miller Publishing, Inc., Minnetonka, MN. 25-33.

Callaway, T. R., C. H. Stahl, T. S. Edrington, K. J. Genovese, L. M. Lincoln, R. C. Anderson, S. M. Lonergan, T. L. Poole, R. B. Harvey and D. J. Nisbet. 2004d. Colicin concentrations inhibit growth of *Escherichia coli* O157:H7 *in vitro*. J. Food Prot. 67:2603-2607.

CDC. 2006. *Salmonella* Annual Summary, 2005. www.cdc.gov/ncidod/dbmd/phlisdata/salmtab/2005/*Salmonella*Table1_2005.pdf 18 June 2007, Accessed.

Coffey, B., L. Rivas, G. Duffy, A. Coffey, R. P. Ross and O. McAuliffe. 2011. Assessment of *Escherichia coli* O157:H7-specific bacteriophages e11/2 and e4/1c in model broth and hide environments. Int. J. Food Microbiol. 147:188-194.

Collins, D. M. and G. R. Gibson. 1999. Probiotics, prebiotics, and synbiotics: approaches for modulating the microbial ecology of the gut. Amer. J. Clin. Nutr. 69:1052S-1057S.

Cornick, N. A. 2010. Tylosin and chlorotetracycline decrease the duration of fecal shedding of *E. coli* O157:H7 by swine. Vet. Microbiol. 143:417-419.

Cull, C. A., Z. D. Paddock, T. G. Nagaraja, N. M. Bello, A. H. Babcock and D. G. Renter. 2012. Efficacy of a vaccine and a direct-fed microbial against fecal shedding of *Escherichia coli* O157:H7 in a randomized pen-level field trial of commercial feedlot cattle. Vaccine. 30:6210-6215.

Cutler, S. A., S. M. Lonergan, N. Cornick, A. K. Johnson and C. H. Stahl. 2007. Dietary inclusion of colicin E1 is effective in preventing postweaning diarrhea caused by F18-positive *Escherichia coli* in pigs. Antimicrob. Ag. Chemother. 51:3830-3835.

Edrington, T. S., T. R. Callaway, R. C. Anderson, K. J. Genovese, Y. S. Jung, R. O. Elder, K. M. Bischoff and D. J. Nisbet. 2003. Reduction of *E. coli* O157:H7 populations in sheep by supplementation of an experimental sodium chlorate product. Small Ruminant Res. 49:173-181.

Elam, N. A., J. F. Gleghorn, J. D. Rivera, M. L. Galyean, P. J. Defoor, M. M. Brashears and S. M. Younts-Dahl. 2003. Effects of live cultures of *Lactobacillus acidophilus* (strains NP45 and NP51) and *Propionibacterium freudenreichii* on performance, carcass, and intestinal characteristics, and *Escherichia coli* strain O157 shedding of finishing beef steers. J. Anim. Sci. 81:2686-2698.

Elder, R. O., J. E. Keen, T. E. Wittum, T. R. Callaway, T. S. Edrington, R. C. Anderson and D. J. Nisbet. 2002. Intervention to reduce fecal shedding of enterohemorrhagic *Escherichia coli* O157:H7 in

naturally infected cattle using neomycin sulfate. J. Anim. Sci. 80 (Suppl. 1):15 (Abstr.).

Fiume, M. Z. 1995. Final report on the safety assessment of potassium chlorate. J. Amer. Coll. Toxicol. 14:221-230.

Fox, J. T., J. S. Drouillard and T. G. Nagaraja. 2009a. Competitive exclusion *Escherichia coli* cultures on *E. coli* O157 growth in batch culture ruminal or fecal microbial fermentation. Foodborne Path. Dis. 6:193-199.

Fox, J. T., D. U. Thomson, J. S. Drouillard, A. B. Thornton, D. T. Burkhardt, D. A. Emery and T. G. Nagaraja. 2009b. Efficacy of *Escherichia coli* O157:H7 siderophore receptor/porin proteins-based vaccine in feedlot cattle naturally shedding *E. coli* O157. Foodborne Path. Dis. 6:893-899.

Fuller, R. 1989. Probiotics in man and animals. J. Appl. Bacteriol. 66:365-378.

Goodridge, L. D. 2008. Phages, bacteria, and food. In: Abedon, S. T. Ed. Bacteriophage Ecology. Nova Publishers, New York. 302-331.

Goodridge, L. D. 2010. Designing phage therapeutics. Curr. Pharmaceut. Biotechnol. 11:15-27.

Gyles, C. L. 1998. Vaccines and shiga toxin-producing *Escherichia coli* in animals. In: Kaper, J. B. and A. D. O'Brien Ed. *Escherichia coli* O157:H7 and other shiga toxin-producing *E. coli* strains. Amer. Soc. Microbiol. Press, Washington, DC. 434-444.

Houdijk, J. G. M., M. W. Bosch, M. W. A. Verstegen and H. J. Berenpas. 1998. Effects of dietary oligosaccharides on the growth and faecal characteristics of young growing pigs. Anim. Feed Sci. Technol. 71:35-48.

Huff, W. E., G. R. Huff, N. C. Rath, J. M. Balog, H. Xie, P. A. Moore and A. M. Donoghue. 2002. Prevention of *Escherichia coli* respiratory infection in broiler chickens with bacteriophage (SPR02). Poult. Sci. 81:437-441.

Jack, R. W., J. R. Tagg and B. Ray. 1995. Bacteriocins of gram-positive bacteria. Microbiol. Rev. 59:171-200.

Joerger, R. D. 2003. Alternatives to antibiotics: Bacteriocins, antimicrobial peptides and bacteriophages. Poult. Sci. 82:640-647.

Johansen, M., L. O. Andresen, L. K. Thomsen, M. E. Busch, H. Wachmann, S. E. Jorsal and C. L. Gyles. 2000. Prevention of edema disease in pigs by passive immunization. Can. J. Vet. Res. 64:9-14.

Johnson, R. P., C. L. Gyles, W. E. Huff, S. Ojha, G. R. Huff, N. C. Rath and A. M. Donoghue. 2008. Bacteriophages for prophylaxis and therapy in cattle, poultry and pigs. Anim. Health Res. Rev. 9:201-215.

Jordi, B. J. A. M., K. Boutaga, C. M. E. Van Heeswijk, F. Van Knapen and L. J. A. Lipman. 2001. Sensitivity of shiga toxin-producing *Escherichia coli* (STEC) strains for colicins under different experimental conditions. FEMS Microbiol. Lett. 204:329-344.

Karesh, W. B., A. Dobson, J. O. Lloyd-Smith, J. Lubroth, M. A. Dixon, M. Bennett, S. Aldrich, T. Harrington, P. Formenty, E. H. Loh, C. C. Machalaba, M. J. Thomas and D. L. Heymann. 2012. Ecology of zoonoses: natural and unnatural histories. Lancet. 380:1936-1945.

Karmali, M. A., V. Gannon and J. M. Sargeant. 2010. Verocytotoxin-producing *Escherichia coli* (VTEC). Vet. Microbiol. 140:360-370.

Keen, J. and R. Elder. 2000. Commercial probiotics are not effective for short-term control of enterohemorrhagic *Escherichia coli* O157 infection in beef cattle. In: Proc.s 4th Intl. Symp. Works. Shiga Toxin (Verocytotoxin)-producing *Escherichia coli* Infect., Kyoto, Japan. 92 (Abstr.).

Khare, S., W. Alali, S. Zhang, D. Hunter, R. Pugh, F. C. Fang, S. J. Libby and L. G. Adams. 2010. Vaccination with attenuated *Salmonella enterica* Dublin expressing *E. coli* O157:H7 outer membrane protein Intimin induces transient reduction of fecal shedding of *E. coli* O157:H7 in cattle. BMC Vet. Res. 6:35-47.

Kim, Y., S. H. Kim, K. Y. Whang, Y. J. Kim and S. Oh. 2008. Inhibition of *Escherichia coli* O157:H7 attachment by interactions between lactic acid bacteria and intestinal epithelial cells. J. Microbiol. Biotechnol. 18:1278-1285.

Kingsley, R. A. and A. J. Bäumler. 2000. Host adaptation and the emergence of infectious disease: The *Salmonella* paradigm. Molec. Microbiol. 36:1006-1014.

Konisky, J. 1982. Colicins and other bacteriocins

with established modes of action. Ann. Rev. Microbiol. 36:125-144.

Kropinski, A. M., E. J. Lingohr, D. M. Moyles, H. W. Ackermann, S. Ojha, A. Mazzocco, Y. M. She, S. J. Bach, E. A. Rozema, K. Stanford, T. A. McAllister and R. P. Johnson. 2012. Endemic bacteriophages: A cautionary tale for evaluation of bacteriophage therapy and other interventions for infection control in animals. Virol. J. 9:207-216.

Kudva, I. T., S. Jelacic, P. I. Tarr, P. Youderian and C. J. Hovde. 1999. Biocontrol of *Escherichia coli* O157 with O157-specific bacteriophages. Appl. Environ. Microbiol. 65:3767-3773.

Kutter, E. and A. Sulakvelidze. 2005. Bacteriophages: Biology and Applications. CRC Press, New York.

Lakey, J. H. and S. L. Slatin. 2001. Pore-forming colicins and their relatives. In: Van Der Goot, F. G. Ed. Pore-forming toxins. Springer-Verlag GmbH & Co. KG, Berlin. pp. 131-161.

Law, D. 2000. The history and evolution of *Escherichia coli* O157 and other shiga toxin-producing *E. coli*. World J. Microbiol. Biotechnol. 16:701-709.

Leatham, M. P., S. Banerjee, S. M. Autieri, R. Mercado-Lubo, T. Conway and P. S. Cohen. 2009. Precolonized human commensal *Escherichia coli* strains serve as a barrier to *E. coli* O157:H7 growth in the streptomycin-treated mouse intestine. Infect. Immun. 77:2876-2886.

Lederberg, J. 1996. Smaller Fleas...ad infinitum: Therapeutic bacteriophage redux. Proc. Natl. Acad. Sci. USA. 93:3167-3168.

LeJeune, J. and A. Kersting. 2010. Zoonoses: An occupational hazard for livestock workers and a public health concern for rural communities. J. Agric. Safe. Health. 16:161-179.

LeJeune, J. T. and A. N. Wetzel. 2007. Preharvest control of *Escherichia coli* O157 in cattle. J. Anim. Sci. 85:e73-80.

Lema, M., L. Williams and D. R. Rao. 2001. Reduction of fecal shedding of enterohemorrhagic *Escherichia coli* O157:H7 in lambs by feeding microbial feed supplement. Small Rum. Res. 39:31-39.

Liu, S. M., D. M. Miller and R. F. Roberts. 2011. Cloning of genes encoding colicin E2 in *Lactococcus lactis* subspecies lactis and evaluation of the colicin-producing transformants as inhibitors of *Escherichia coli* O157:H7 during milk fermentation. J. Dairy Sci. 94:1146-1154.

Liu, Y., J. H. Li, H. Y. Shi, J. P. Wen and Y. B. Sun. 2012. Biological characteristics of enterohemorrhagic *E. coli* O157-specific bacteriophages isolated from raw sewage. J. Jilin Univ. Med. Ed. 38:79-83.

López-Cuevas, O., N. Castro-del Campo, J. León-Félix, A. González-Robles and C. Chaidez. 2011. Characterization of bacteriophages with a lytic effect on various *Salmonella* serotypes and *Escherichia coli* O157:H7. Can. J. Microbiol. 57:1042-1051.

López-Cuevas, O., N. Castro-Del Campo, J. León-Félix, B. Valdez-Torres and C. Chaidez. 2012. Evaluation of bacteriophage av-08 for simultaneous biocontrol of *Salmonella* Montevideo and *Escherichia coli* O157: H7 in experimentally contaminated chicken skin. J. Food Safety 32:305-310.

Loretz, M., R. Stephan and C. Zweifel. 2011. Antibacterial activity of decontamination treatments for cattle hides and beef carcasses. Food Control. 22:347-359.

Martin, S. A. and D. J. Nisbet. 1992. Effect of direct-fed microbials on rumen microbial fermentation. J. Dairy Sci. 75:1736-1744.

Mayr, U. B., P. Kudela, A. Atrasheuskaya, E. Bukin, G. Ignatyev and W. Lubitz. 2012. Rectal single dose immunization of mice with *Escherichia coli* O157: H7 bacterial ghosts induces efficient humoral and cellular immune responses and protects against the lethal heterologous challenge. Microb. Biotechnol. 5:283-294.

McAllister, T. A., K. A. Beauchemin, A. Y. Alazzeh, J. Baah, R. M. Teather and K. Stanford. 2011. Review: The use of direct fed microbials to mitigate pathogens and enhance production in cattle. Can. J. Anim. Sci. 91:193-211.

McLaughlin, M. R., M. F. Balaa, J. Sims and R. King. 2006. Isolation of *Salmonella* bacteriophages from swine effluent lagoons. J. Environ. Qual. 35:522-528.

McNeilly, T. N., M. C. Mitchell, T. Rosser, S. McAteer, J. C. Low, D. G. E. Smith, J. F. Huntley, A. Mahajan

and D. L. Gally. 2010. Immunization of cattle with a combination of purified intimin-531, EspA and Tir significantly reduces shedding of *Escherichia coli* O157:H7 following oral challenge. Vaccine. 28:1422-1428.

Moxley, R. A., D. Smith, T. J. Klopfenstein, G. Erickson, J. Folmer, C. Macken, S. Hinkley, A. Potter and B. Finlay. 2003. Vaccination and feeding a competitive exclusion product as intervention strategies to reduce the prevalence of *Escherichia coli* O157:H7 in feedlot cattle. In: Proc.s Proc. 5th Int. Symp. on Shiga Toxin-Producing *Escherichia coli* Infections, Edinburgh, UK. 23 (Abstr.).

Moxley, R. A., D. R. Smith, M. Luebbe, G. E. Erickson, T. J. Klopfenstein and D. Rogan. 2009. *Escherichia coli* O157:H7 vaccine dose-effect in feedlot cattle. Foodborne Path. Dis. 6:879-884.

Murinda, S. E., S. M. Liu, R. F. Roberts and R. A. Wilson. 1998. Colicinogeny among *Escherichia coli* serotypes, including O157:H7, representing four closely related diarrheagenic clones. J. Food Prot. 61:1431-1438.

Murinda, S. E., R. F. Roberts and R. A. Wilson. 1996. Evaluation of colicins for inhibitory activity against diarrheagenic *Escherichia coli* strains, including serotype O157:H7. Appl. Environ. Microbiol. 62:3192-3202.

Nandiwada, L. S., G. P. Schamberger, H. W. Schafer and F. Diez-Gonzalez. 2004. Characterization of an E2-type colicin and its application to treat alfalfa seeds to reduce *Escherichia coli* O157:H7. Int. J. Food Microbiol. 93:267-279.

Niu, Y. D., T. A. McAllister, Y. Xu, R. P. Johnson, T. P. Stephens and K. Stanford. 2009. Prevalence and impact of bacteriophages on the presence of *Escherichia coli* O157:H7 in feedlot cattle and their environment. Appl. Environ. Microbiol. 75:1271-1278.

Niu, Y. D., K. Stanford, H. W. Ackermann and T. A. McAllister. 2012. Characterization of 4 T1-like lytic bacteriophages that lyse Shiga-toxin *Escherichia coli* O157:H7. Can. J. Microbiol. 58:923-927.

Niu, Y. D., Y. Xu, T. A. McAllister, E. A. Rozema, T. P. Stephens, S. J. Bach, R. P. Johnson and K. Stanford. 2008. Comparison of fecal versus rectoanal mucosal swab sampling for detecting *Escherichia coli* O157:H7 in experimentally inoculated cattle used in assessing bacteriophage as a mitigation strategy. J. Food Prot. 71:691-698.

Nurmi, E., L. Nuotio and C. Schncitz. 1992. The competitive exclusion concept: development and future. Int. J. Food Microbiol. 15:237-240.

Ohya, T., M. Akiba and H. Ito. 2001. Use of a trial probiotic product in calves experimentally infected with *Escherichia coli* O157. Japan Agric. Res. Quart. 35:189-194.

Oliver, S. P., D. A. Patel, T. R. Callaway and M. E. Torrence. 2008. ASAS Centennial Paper: Developments and future outlook for preharvest food safety. J. Anim. Sci. 87:419-437.

Oot, R. A., R. R. Raya, T. R. Callaway, T. S. Edrington, E. M. Kutter and A. D. Brabban. 2007. Prevalence of *Escherichia coli* O157 and O157:H7-infecting bacteriophages in feedlot cattle feces. Lett. Appl. Microbiol. 45:445-453.

Park, M., J. H. Lee, H. Shin, M. Kim, J. Choi, D. H. Kang, S. Heu and S. Ryu. 2012. Characterization and comparative genomic analysis of a novel bacteriophage, SFP10, simultaneously inhibiting both *Salmonella enterica* and *Escherichia coli* O157:H7. Appl. Environ. Microbiol. 78:58-69.

Patterson, J. A. and K. M. Burkholder. 2003. Application of prebiotics and probiotics in poultry production. Poult. Sci. 82:627-631.

Patton, B. S., S. M. Lonergan, S. A. Cutler, C. H. Stahl and J. S. Dickson. 2008. Application of colicin E1 as a prefabrication intervention strategy. J. Food Prot. 71:2519-2522.

Potter, A. A., S. Klashinsky, Y.Li, E. Frey, H. Townsend, D. Rogan, G. Erickson, S. Hinkley, T. Klopfenstein, R. A. Moxley, D. R. Smith and B. B. Finlay. 2004. Decreased shedding of *Escherichia coli* O157:H7 by cattle following vaccination with type III secreted proteins. Vaccine. 22:362-369.

Rabinovitz, B. C., E. Gerhardt, C. Tironi Farinati, A. Abdala, R. Galarza, D. A. Vilte, C. Ibarra, A. Cataldi and E. C. Mercado. 2012. Vaccination of pregnant cows with EspA, EspB, -intimin, and Shiga toxin 2 proteins from *Escherichia coli* O157: H7 induces high levels of specific colostral antibodies that

are transferred to newborn calves. J. Dairy Sci. 95:3318-3326.

Raya, R. R., P. Varey, R. A. Oot, M. R. Dyen, T. R. Callaway, T. S. Edrington, E. M. Kutter and A. D. Brabban. 2006. Isolation and characterization of a new T-even bacteriophage, CEV1, and determination of its potential to reduce *Escherichia coli* O157:H7 levels in sheep. Appl. Environ. Microbiol. 72:6405-6410.

Reissbrodt, R., W. P. Hammes, F. Dal Bello, R. Prager, A. Fruth, K. Hantke, A. Rakin, M. Starcic-Erjavec and P. H. Williams. 2009. Inhibition of growth of Shiga toxin-producing *Escherichia coli* by nonpathogenic *Escherichia coli*. FEMS Microbiol. Lett. 290:62-69.

Ricke, S. C. 2003. Perspectives on the use of organic acids and short chain fatty acids as antimicrobials. Poult. Sci. 82:632-639.

Ricke, S. C., P. Hererra and D. Biswas. 2012. Bacteriophages for potential food safety applications in organic meat production. In: S. C. Ricke, E. J. Van Loo, M. G. Johnson and C. A. O'Bryan Ed. Organic meat production and processing. John Wiley & Sons, Inc., New York. 407-424.

Rivas, L., B. Coffey, O. McAuliffe, M. J. McDonnell, C. M. Burgess, A. Coffey, R. P. Ross and G. Duffy. 2010. *In vivo* and ex vivo evaluations of bacteriophages e11/2 and e4/1c for use in the control of *Escherichia coli* O157:H7. Appl. Environ. Microbiol. 76:7210-7216.

Rodriguez-Palacios, A., H. R. Staempfli, T. Duffield and J. S. Weese. 2009. Isolation of bovine intestinal *Lactobacillus plantarum* and *Pediococcus acidilactici* with inhibitory activity against *Escherichia coli* O157 and F5. J. Appl. Microbiol. 106:393-401.

Rotariu, O., I. Ogden, L. MacRitchie, K. Forbes, A. Williams, P. Cross, C. Hunter, P. Teunis and N. Strachan. 2012. Combining risk assessment and epidemiological risk factors to elucidate the sources of human *E. coli* O157 infection. Epidemiol. Infect. 140:1414-1429.

Rozema, E. A., T. P. Stephens, S. J. Bach, E. K. Okine, R. P. Johnson, K. I. M. Stanford and T. A. McAllister. 2009. Oral and rectal administration of bacteriophages for control of *Escherichia coli* O157:H7 in feedlot cattle. J. Food Prot. 72:241-250.

Russell, J. B. 1992. Another explanation for the toxicity of fermentation acids at low pH: anion accumulation versus uncoupling. J. Appl. Bacteriol. 73:363-370.

Sargeant, J. M., M. R. Amezcua, A. Rajic and L. Waddell. 2007. Pre-harvest interventions to reduce the shedding of *E. coli* O157 in the faeces of weaned domestic ruminants: a systematic review. Zoonos. Pub. Health. 54:260-277.

Sawant, A. A., N. C. Casavant, D. R. Call and T. E. Besser. 2011. Proximity-dependent inhibition in *Escherichia coli* isolates from cattle. Appl. Environ. Microbiol. 77:2345-2351.

Scallan, E., R. M. Hoekstra, F. J. Angulo, R. V. Tauxe, M.-A. Widdowson, S. L. Roy, J. L. Jones and P. L. Griffin. 2011. Foodborne illness acquired in the United States—major pathogens. Emerg. Infect. Dis. 17:7-15.

Schamberger, G. P. and F. Diez-Gonzalez. 2002. Selection of recently isolated colicinogenic *Escherichia coli* strains inhibitory to *Escherichia coli* O157:H7. J. Food Prot. 65:1381-1387.

Schamberger, G. P., R. L. Phillips, J. L. Jacobs and F. Diez-Gonzalez. 2004. Reduction of *Escherichia coli* O157:H7 populations in cattle by addition of colicin E7-producing *E. coli* to feed. Appl. Environ. Microbiol. 70:6053-6060.

Scharff, R. L. 2010. Health-related costs from foodborne illness in the United States. http://www.publichealth.lacounty.gov/eh/docs/ReportPublication/HlthRelatedCostsFromFoodborneIllinessUS.pdf. 3 May, Accessed 2010.

Schmidt, J. W., R. Wang, N. Kalchayanand, T. L. Wheeler and M. Koohmaraie. 2012. Efficacy of hypobromous acid as a hide-on carcass antimicrobial intervention. J. Food Prot. 75:955-958.

Schrezenmeir, J. and M. De Vrese. 2001. Probiotics, prebiotics, and synbiotics-approaching a definition. Am. J. Clin. Nutr. 73(Suppl.):354s-361s.

Sharma, V. K., E. A. Dean-Nystrom and T. A. Casey. 2011. Evaluation of *hha* and *hha sepB* mutant strains of *Escherichia coli* O157:H7 as bacterins for reducing *E. coli* O157:H7 shedding in cattle. Vaccine. 29:5078-5086.

Siragusa, G. R. and S. C. Ricke. 2012. Probiotics as pathogen control agents for organic meat production. In: S. C. Ricke, E. J. Van Loo, M. G. Johnson and C. A. O'Bryan Ed. Organic meat production and processing. John Wiley & Sons, Inc., New York. 331-349.

Smarda, J. and D. Smajs. 1998. Colicins- Exocellular lethal proteins of *Escherichia coli*. Folia Microbiol. 43:563-582.

Smith, D. R., R. A. Moxley, T. J. Klopfenstein and G. E. Erickson. 2009a. A randomized longitudinal trial to test the effect of regional vaccination within a cattle feedyard on *Escherichia coli* O157:H7 rectal colonization, fecal shedding, and hide contamination. Foodborne Path. Dis. 6:885-892.

Smith, D. R., R. A. Moxley, R. E. Peterson, T. J. Klopfenstein, G. E. Erickson, G. Bretschneider, E. M. Berberov and S. Clowser. 2009b. A two-dose regimen of a vaccine against type III secreted proteins reduced *Escherichia coli* O157:H7 colonization of the terminal rectum in beef cattle in commercial feedlots. Foodborne Path. Dis. 6:155-161.

Smith, H. W. and R. B. Huggins. 1982. Successful treatment of experimental *E. coli* infections in mice using phage: its general superiority over antibiotics. J. Gen. Microbiol. 128:307-318.

Smith, H. W. and R. B. Huggins. 1983. Effectiveness of phages in treating experimental *Escherichia coli* diarrhoea in calves, piglets and lambs. J. Gen. Microbiol. 129:2659-2675.

Smith, H. W. and R. B. Huggins. 1987. The control of experimental *E. coli* diarrhea in calves by means of bacteriophage. J. Gen. Microbiol. 133:1111-1126.

Snedeker, K. G., M. Campbell and J. M. Sargeant. 2012. A systematic review of vaccinations to reduce the shedding of *Escherichia coli* O157 in the faeces of domestic ruminants. Zoono. Pub. Health. 59:126-138.

Stahl, C. H., T. R. Callaway, L. M. Lincoln, S. M. Lonergan and K. J. Genovese. 2004. Inhibitory activities of colicins against *Escherichia coli* strains responsible for postweaning diarrhea and edema disease in swine. Antimicrob. Ag. Chemother. 48:3119-3121.

Stanford, K., T. A. McAllister, Y. D. Niu, T. P. Stephens, A. Mazzocco, T. E. Waddell and R. P. Johnson. 2010. Oral delivery systems for encapsulated bacteriophages targeted *Escherichia coli* O157: H7 in feedlot cattle. J. Food Prot. 73:1304-1312.

Stephens, T. P., G. H. Loneragan, L. M. Chichester and M. M. Brashears. 2007a. Prevalence and enumeration of *Escherichia coli* O157 in steers receiving various strains of *Lactobacillus*-based direct-fed microbials. J. Food Prot. 70:1252-1255.

Stephens, T. P., G. H. Loneragan, E. Karunasena and M. M. Brashears. 2007b. Reduction of *Escherichia coli* O157 and *Salmonella* in feces and on hides of feedlot cattle using various doses of a direct-fed microbial. J. Food Prot. 70:2386-2391.

Stewart, V. J. 1988. Nitrate respiration in relation to facultative metabolism in enterobacteria. Microbiol. Rev. 52:190-232.

Stouthamer, A. H. 1969. A genetical and biochemical study of chlorate-resistant mutants of *Salmonella typhimurium*. Antoine van Leeuwenhoek. 35:505-521.

Swyers, K. L., B. A. Carlson, K. K. Nightingale, K. E. Belk and S. L. Archibeque. 2011. Naturally colonized beef cattle populations fed combinations of yeast culture and an ionophore in finishing diets containing dried distiller's grains with solubles had similar fecal shedding of *Escherichia coli* O157:H7. J. Food Prot. 74:912-918.

Tanji, Y., T. Shimada, H. Fukudomi, K. Miyanaga, Y. Nakai and H. Unno. 2005. Therapeutic use of phage cocktail for controlling *Escherichia coli* O157:H7 in gastrointestinal tract of mice. J. Biosci. Bioeng. 100:280-287.

Thomson, D. U., G. H. Loneragan, A. B. Thornton, K. F. Lechtenberg, D. A. Emery, D. T. Burkhardt and T. G. Nagaraja. 2009. Use of a siderophore receptor and porin proteins-based vaccine to control the burden of *Escherichia coli* O157:H7 in feedlot cattle. Foodborne Path. Dis. 6:871-877.

Thornton, A. B., D. U. Thomson, G. H. Loneragan, J. T. Fox, D. T. Burkhardt, D. A. Emery and T. G. Nagaraja. 2009. Effects of a siderophore receptor and porin proteins-based vaccination on fecal shedding of *Escherichia coli* O157:H7 in experimentally inoculated cattle. J. Food Prot. 72:866-869.

Tournut, J. 1989. Applications of probiotics to animal husbandry. Rev. Sci. Tech. Off. Int. Epiz. 8:551-566.

Van Donkersgoed, J., D. Hancock, D. Rogan and A. A. Potter. 2005. *Escherichia coli* O157:H7 vaccine field trial in 9 feedlots in Alberta and Saskatchewan. Can. Vet. J. 46:724-728.

Varela, N. P., P. Dick and J. Wilson. 2013. Assessing the existing information on the efficacy of bovine vaccination against *Escherichia coli* O157: H7 - a systematic review and meta-analysis. Zoonos. Pub. Health. 60:253-268.

Vilte, D. A., M. Larzábal, U. B. Mayr, S. Garbaccio, M. Gammella, B. C. Rabinovitz, F. Delgado, V. Meikle, R. J. C. Cantet, P. Lubitz, W. Lubitz, A. Cataldi and E. C. Mercado. 2012. A systemic vaccine based on *Escherichia coli* O157:H7 bacterial ghosts (BGs) reduces the excretion of *E. coli* O157:H7 in calves. Vet. Immunol. Immunopathol. 146:169-176.

Walker, W. A. and L. C. Duffy. 1998. Diet and bacterial colonization: Role of probiotics and prebiotics. J. Nutr. Biochem. 9:668-675.

Walle, K. V., D. Vanrompay and E. Cox. 2012. Bovine innate and adaptive immune responses against *Escherichia coli* O157:H7 and vaccination strategies to reduce faecal shedding in ruminants. Vet. Immunol. Immunopathol. 152:109-120.

Wileman, B. W., D. U. Thomson, K. C. Olson, J. R. Jaeger, L. A. Pacheco, J. Bolte, D. T. Burkhardt, D. A. Emery and D. Straub. 2011. *Escherichia coli* O157:H7 shedding in vaccinated beef calves born to cows vaccinated prepartum with *Escherichia coli* O157:H7 SRP vaccine. J. Food Prot. 74:1599-1604.

Willard, M. D., R. B. Simpson, N. D. Cohen and J. S. Clancy. 2000. Effects of dietary fructooligosaccharide on selected bacterial populations in feces of dogs. Amer. J. Vet. Res. 61:820-825.

Wolin, M. J. 1969. Volatile fatty acids and the inhibition of *Escherichia coli* growth by rumen fluid. Appl. Microbiol. 17:83-87.

Yekta, M. A., B. M. Goddeeris, D. Vanrompay and E. Cox. 2011. Immunization of sheep with a combination of intiminγ, EspA and EspB decreases *Escherichia coli* O157:H7 shedding. Vet. Immunol. Immunopathol. 140:42-46.

Yoon, I. K. and M. D. Stern. 1996. Effects of *Saccharomyces cerevisiae* and *Aspergillus oryzae* cultures on ruminal fermentation in dairy cows. J. Dairy Sci. 79:411-417.

Younts-Dahl, S. M., M. L. Galyean, G. H. Loneragan, N. A. Elam and M. M. Brashears. 2004. Dietary supplementation with *Lactobacillus*- and Propionibacterium-based direct-fed microbials and prevalence of *Escherichia coli* O157 in beef feedlot cattle and on hides at harvest. J. Food Prot. 67:889-893.

Zhang, X. H., K. W. He, P. D. Zhao, Q. Ye, X. T. Luan, Z. Y. Yu, L. B. Wen, Y. X. Ni, B. Li, X. M. Wang, R. L. Guo, J. M. Zhou and A. H. Mao. 2012. Intranasal immunisation with Stx2B-Tir-Stx1B-Zot protein leads to decreased shedding in goats after challenge with *Escherichia coli* O157:H7. Vet. Rec. 170:178-186.

Zhao, T., M. P. Doyle, B. G. Harmon, C. A. Brown, P. O. E. Mueller and A. H. Parks. 1998. Reduction of carriage of enterohemorrhagic *Escherichia coli* O157:H7 in cattle by inoculation with probiotic bacteria. J. Clin. Microbiol. 36:641-647.

Zhao, T., S. Tkalcic, M. P. Doyle, B. G. Harmon, C. A. Brown and P. Zhao. 2003. Pathogenicity of enterohemorrhagic *Escherichia coli* in neonatal calves and evaluation of fecal shedding by treatment with probiotic *Escherichia coli*. J. Food Prot. 66:924-930.

Zopf, D. and S. Roth. 1996. Oligosaccharide anti-infective agents. The Lancet (North America). 347:1017-1021.