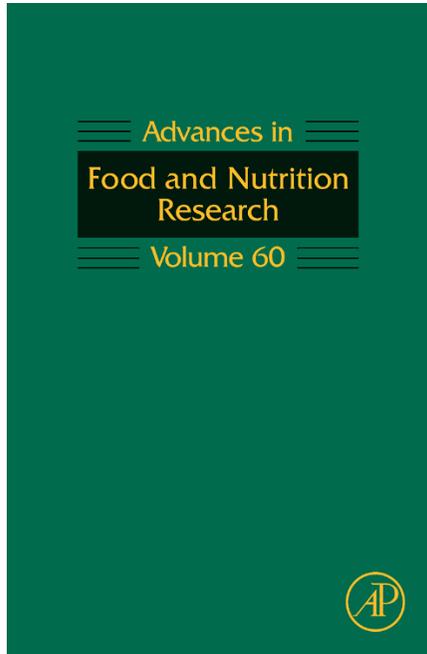


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CHAPTER 4

Escherichia coli O157:H7: Recent Advances in Research on Occurrence, Transmission, and Control in Cattle and the Production Environment

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Abstract

Escherichia coli O157:H7 is a zoonotic pathogen that is an important cause of human foodborne and waterborne disease, with a spectrum of illnesses ranging from asymptomatic carriage and diarrhea to the sometimes fatal hemolytic uremic syndrome. Outbreaks of *E. coli* O157:H7 disease are often associated with undercooked beef, but there are other sources of transmission, including water, produce, and animal contact, which can often be linked directly or indirectly to cattle. Thus, preharvest control of this pathogen in cattle production should have a large impact on reducing the risk of human foodborne illness. In this review, we will summarize preharvest research on *E. coli* O157:H7 in cattle and the production environment, focusing on factors that may influence the transmission, prevalence, and levels of this pathogen, such as season, diet, high-level shedders, and animal stress. In addition, we will discuss recent research on the reduction of this pathogen in cattle production, including vaccination, probiotics, bacteriophage, and manure treatments.

I. INTRODUCTION

Escherichia coli O157:H7 became recognized as a human pathogen and a cause of foodborne disease in 1982, following two outbreaks of hemorrhagic colitis linked to the consumption of hamburgers (Riley *et al.*, 1983; Wells *et al.*, 1983). Increased surveillance following these discoveries has identified *E. coli* O157:H7 as an important cause of bacterial diarrhea, and that the spectrum of illnesses caused by this pathogen include asymptomatic carriage, nonbloody diarrhea, hemorrhagic colitis, thrombotic thrombocytopenic purpura, and hemolytic uremic syndrome (Cohen and Giannella, 1992; Griffin *et al.*, 1988; Su and Brandt, 1995). Children and the elderly are particularly at risk for infection and for the associated complications of thrombotic thrombocytopenic purpura, hemolytic uremic syndrome, and death (Cohen and Giannella, 1992; Griffin *et al.*, 1988; Reiss *et al.*, 2006; Su and Brandt, 1995). The Centers for Disease Control and Prevention has estimated that *E. coli* O157:H7 causes 73,480 cases of human illness annually, including 2168 hospitalizations and 61 deaths (Mead *et al.*, 1999).

Human disease caused by *E. coli* O157:H7 is often associated with contaminated and undercooked beef (Bell *et al.*, 1994; CDC, 1997; Riley *et al.*, 1983; Rodrigue *et al.*, 1995; Slutsker *et al.*, 1998). However, improved outbreak surveillance for this organism has revealed many other modes of transmission of *E. coli* O157:H7 to humans, many of which can be linked directly or indirectly to cattle. Other bovine-derived food vehicles include unpasteurized milk (Bhat *et al.*, 2007; Keene *et al.*, 1997), raw milk cheese and butter (Rangel *et al.*, 2005), and dry-cured salami (CDC, 1995). Produce has become a common source of foodborne *E. coli* O157:H7 since the first U.S. outbreaks were reported in 1991 (Rangel *et al.*, 2005); produce items associated with outbreaks include spinach, lettuces, apple cider and juice, coleslaw, and sprouts (Besser *et al.*, 1993; CDC, 2006; Hilborn *et al.*, 1999; Rangel *et al.*, 2005). Both drinking water and recreational waters have been the sources of *E. coli* O157:H7 that caused human illness outbreaks (Bruce *et al.*, 2003; O'Connor, 2002; Swerdlow *et al.*, 1992). In 2000, an estimated 2300 people became ill and seven died in a large waterborne outbreak in Walkerton, Ontario, Canada, that was caused by both *E. coli* O157:H7 and *Campylobacter jejuni* contamination of the municipal water supply by runoff from land-applied bovine manure (O'Connor, 2002). Numerous *E. coli* O157:H7 outbreaks resulting from contact with cattle or their manure on farms, at fairs, and at petting zoos have also been reported (CDC, 2005; Durso *et al.*, 2005; Rangel *et al.*, 2005).

Research on *E. coli* O157:H7 in cattle intensified following a large multistate outbreak in 1993 that was due to the consumption of undercooked hamburgers (Bell *et al.*, 1994). Early work established that cattle are an important reservoir of this pathogen and that there is a seasonal pattern of shedding, but indicated that prevalence of *E. coli* O157:H7 was low, being typically less than 5.0% (Hancock *et al.*, 1994, 1997; Wells *et al.*, 1991). However, with the development and use of more sensitive isolation and detection procedures (in particular immunomagnetic separation; Chapman *et al.*, 1994), it was found that prevalence of the pathogen is often much higher than previously thought. Van Donkersgoed *et al.* (1999) reported *E. coli* O157:H7 in 7.5% of feces samples collected from cattle at slaughter, with a peak prevalence rate of 19.7% during the months of June to August. Similarly, Elder *et al.* (2000) isolated *E. coli* O157 from 28% of feces samples collected from fed beef cattle at slaughter during July and August. In a subsequent study examining fed beef cattle presented for slaughter at three Midwestern U.S. processing plants, Barkocy-Gallagher *et al.* (2003) observed a peak *E. coli* O157:H7 fecal prevalence rate of 12.9% in the summer months, in comparison with 6.8%, 0.3%, and 3.9% in the fall, winter, and spring months. Rhoades *et al.* (2009) recently compiled published data on the prevalence of Shiga toxin-producing *E. coli* in feces and at other points in beef production.

E. coli O157:H7 fecal shedding and hide prevalence are correlated with beef carcass contamination (Arthur *et al.*, 2004; Brichta-Harhay *et al.*, 2008; Elder *et al.*, 2000), indicating that control strategies targeted at the live animal should reduce the risk of illness associated with undercooked beef consumption. This is further suggested by model simulations assessing the benefits of preharvest control measures to reduce *E. coli* O157:H7 contamination of beef carcasses (Jordan *et al.*, 1999). In addition, reducing the load of this pathogen in and on cattle should enhance the effectiveness of postharvest carcass intervention efforts (Brichta-Harhay *et al.*, 2008). The various vehicles and routes by which *E. coli* O157:H7 is transmitted to humans clearly demonstrate that the problem of *E. coli* O157:H7 is not confined to beef products, and further suggest that preharvest control of this microorganism in cattle production should have a large impact on the reduction of risk of illness from water, produce, and other environmental sources. Finally, it is important to note that there are a number of other non-O157 Shiga-toxicogenic *E. coli* (non-O157 STEC) that are important human pathogens that have been linked to cattle and bovine food products (Bettelheim, 2007). As reviewed by Bettelheim (2007), the inability of most *E. coli* O157:H7 to ferment sorbitol has provided a convenient marker for selecting this pathogen; a result of this, at least in part, is that a preponderance of the research on the ecology and distribution of STEC has been focused on *E. coli* O157:H7. This chapter will provide a summary of research on *E. coli* O157:H7 in cattle (or *E. coli* O157, as reported by individual studies), including sources, transmission, and those factors that affect the prevalence and numbers of the pathogen in cattle and the production environment. In addition, we will highlight recent discoveries and review potential strategies for *E. coli* O157:H7 reduction and control.

II. SOURCES AND TRANSMISSION OF *E. COLI* O157:H7 IN CATTLE

Research efforts to understand the on-farm ecology of *E. coli* O157:H7 have found this pathogen in dairy and beef cattle, in calves and adult animals, and in cattle in both feedlot-, confinement- and pasture-based production systems (Gannon *et al.*, 2002; Hancock *et al.*, 1994, 1997; Laegreid *et al.*, 1999; Ogden *et al.*, 2004; Synge *et al.*, 2003; Wells *et al.*, 1991). In addition, *E. coli* O157:H7 has been isolated from feces of both organically raised and naturally raised cattle, and while data yet are limited, there are no apparent differences in the prevalence of this organism in cattle from these niche marketing production systems in comparison with conventionally raised cattle (Fox *et al.*, 2008a; Kuhnert *et al.*, 2005; Reinstein *et al.*, 2009). Numerous nonbovine animals and other sources have been identified as potential reservoirs or vehicles of *E. coli* O157:H7,

including other livestock and domestic animals, many kinds of wild animals and birds, flies, water, feed, and manure. *E. coli* O157:H7 has been isolated from sheep (Chapman *et al.*, 1997; Kudva *et al.*, 1996), goats (Fox *et al.*, 2007a; Keen *et al.*, 2006a), pigs (Chapman *et al.*, 1997; Doane *et al.*, 2007; Feder *et al.*, 2003), horses (Hancock *et al.*, 1998), dogs (Hancock *et al.*, 1998), chickens (Dipineto *et al.*, 2006; Doane *et al.*, 2007), and turkeys (Doane *et al.*, 2007). The prevalence of this pathogen in sheep and goats and links to human illness suggest that these small ruminants may be a significant reservoir of *E. coli* O157:H7 (La Ragione *et al.*, 2009). Kudva *et al.* (1996) reported a peak prevalence of 31% in free-ranging sheep in June. Flock level prevalence of 40% and fecal prevalence of 6.5% was reported for pastured sheep on 15 farms in Scotland, and fecal concentrations as high as 10^4 CFU/g of *E. coli* O157 were observed (Ogden *et al.*, 2005). Keen *et al.* (2006a) isolated *E. coli* O157 from 11% of sheep and 2% of goats at county and state fairs in the United States. Overall *E. coli* O157 prevalence in 181 Boer-sired finishing goats during a 105-day feeding period was 4.6% (Fox *et al.*, 2007a). Transmission of the pathogen to humans via consumption of unpasteurized goat's milk (Bielaszewska *et al.*, 1997; McIntyre *et al.*, 2002), environmental contact with sheep manure (Ogden *et al.*, 2002), and sheep or goat contact at petting zoos (Heuvelink *et al.*, 2002; Payne *et al.*, 2003) has been reported.

Other animals demonstrated to carry *E. coli* O157:H7 include, but are not limited to, both tame and wild deer (Fischer *et al.*, 2001; Heuvelink *et al.*, 2002; Sargeant *et al.*, 1999), rabbits (Scaife *et al.*, 2006), raccoons (Shere *et al.*, 1998), opossums (Renter *et al.*, 2003), and rats (Cizek *et al.*, 1999). Pigeons (Shere *et al.*, 1998), gulls (Wallace *et al.*, 1997), rooks (Ejidokun *et al.*, 2006), and starlings (Nielsen *et al.*, 2004) are among the numerous bird species that have been found positive for *E. coli* O157 and other STEC, leading to speculation about an important role for birds that frequent feedlots and farms in the transfer and dissemination of this pathogen from cattle (Bach *et al.*, 2002c; Wetzel and LeJeune, 2006).

A. Flies

Livestock manure is a favorite developmental site, food source, and landing spot for flies, and flies can subsequently contaminate other surfaces with pathogens from manure by regurgitation, fecal deposition, or mechanical transfer (Graczyk *et al.*, 2001). Several studies have detected *E. coli* O157:H7 in flies collected from both dairy and beef cattle production environments (Alam and Zurek, 2004; Hancock *et al.*, 1998; Iwasa *et al.*, 1999; Shere *et al.*, 1998). Fly species shown to harbor this pathogen include members of the Muscidae and Calliphoridae families; however, houseflies (*Musca domestica*) are most typically implicated (Iwasa *et al.*, 1999; Keen *et al.*, 2006a; Moriya *et al.*, 1999; Talley *et al.*, 2009). Alam and

Zurek (2004) collected houseflies at a Kansas cattle feedlot weekly from June to October. They found an overall *E. coli* O157:H7 prevalence of 2.2% of individual flies, and found levels of *E. coli* O157:H7 as high as 1.5×10^5 CFU per fly. Houseflies were implicated in the transmission of *E. coli* O157:H7 from cattle to humans via food contamination (Moriya *et al.*, 1999), and in laboratory studies, were demonstrated to transfer the pathogen onto spinach leaves (Talley *et al.*, 2009). Ahmad *et al.* (2007) demonstrated that houseflies can transmit *E. coli* O157:H7 to cattle. Similarly, pulsed-field gel electrophoresis patterns of *E. coli* O157:H7 were sometimes indistinguishable in fly and livestock isolates collected at the same U.S. agricultural fairs, indicating transfer of the pathogen (Keen *et al.*, 2006a). The persistence and proliferation of *E. coli* O157:H7 in and on houseflies suggested to Kobayashi *et al.* (1999) that houseflies are more than just mechanical vectors for this pathogen, and that they may play a more significant role in the transmission of this organism in cattle production. The increases in *E. coli* O157:H7 prevalence in cattle during the warmer summer months coincide with increases in fly populations, though a direct impact of flies on *E. coli* O157:H7 prevalence in cattle has not been demonstrated (Rasmussen and Casey, 2001). Flies are ubiquitous in cattle production environments and can generally move about the feedlot or farmyard unencumbered. As a result, if flies had a significant effect on the overall incidence of *E. coli* O157:H7, one might anticipate that the prevalence of the pathogen in cattle would be more homogenous across pens within a feedlot. Instead, a high degree of variation in prevalence across pens typically has been observed (Arthur *et al.*, 2009; LeJeune *et al.*, 2004; Smith *et al.*, 2001; Wells *et al.*, 2009). Thus, while flies can transmit *E. coli* O157:H7, they do not appear to have a large effect on the overall prevalence of this microorganism in cattle.

B. Feed

Animal feed has been suggested to be a vehicle for transmission of *E. coli* O157:H7 to cattle (Hancock *et al.*, 2001; Lynn *et al.*, 1998; Rice *et al.*, 1999). Shere *et al.* (1998) found *E. coli* O157:H7 in 3 of 32 feed samples collected from feed bunks on one of four dairy farms examined; molecular subtyping by pulsed-field gel electrophoresis indicated that the feed isolates were of the same *E. coli* O157:H7 strain as that found in cattle, flies, and water samples on that farm. While cattle on another of the remaining three farms were also shedding *E. coli* O157:H7 during the study, the pathogen was not recovered from feed samples collected on that farm (Shere *et al.*, 1998). Dodd *et al.* (2003) detected *E. coli* O157:H7 in 14.9% of feed samples from feed bunks from 54 Midwestern feedlots. Van Donkersgoed *et al.* (2001) found *E. coli* O157 in 1.7% of feed samples from feed bunks, but not in fresh mixed rations that had been collected

prior to feeding. Similar to the observations of [Shere et al. \(1998\)](#), molecular subtyping of *E. coli* O157:H7 indicated transmission of the pathogen among cattle, water, and feed within the same feedlot ([Van Donkersgoed et al., 2001](#)). [Davis et al. \(2003\)](#) found low prevalences of *E. coli* O157:H7 in feed components (0.2%) and feed mill samples (0.4%). For feed before and after cattle access, [Sanderson et al. \(2006\)](#) reported 1.25% and 3.25% of feed samples positive for *E. coli* O157, respectively. Similarly, [Doane et al. \(2007\)](#) intermittently isolated *E. coli* O157:H7 from feed samples, both before distribution to animals and after placement in feeders or troughs. Conversely, other studies did not find this pathogen in cattle feed ([Hancock et al., 1998](#); [Lynn et al., 1998](#)).

The above studies indicate that recovery of *E. coli* O157:H7 from feeds prior to exposure to cattle is infrequent. Once placed in the feedbunk, feeds may be contaminated with the pathogen by cattle feces or saliva, birds, or other vermin ([Dodd et al., 2003](#)). Thus, while a role for feeds in transmission of *E. coli* O157:H7 among cattle is plausible, a clear role for feed hygiene controls to reduce this pathogen in cattle production is not apparent. This was indicated by [Smith et al. \(2001\)](#), who examined *E. coli* O157:H7 prevalence in more than 3100 cattle in 29 pens of five U.S. feedlots, and found no relationship between the prevalence of fecal shedding and the presence of the pathogen in feed.

C. Water

Numerous studies have demonstrated that cattle drinking water can be a reservoir of *E. coli* O157:H7, and a means of disseminating this pathogen to cattle ([Faith et al., 1996](#); [Hancock et al., 1998](#); [LeJeune et al., 2001a,b](#); [Shere et al., 1998](#); [Van Donkersgoed et al., 2001](#)). *E. coli* O157 was isolated from 3.1% of 320 water trough samples collected at feedlots and dairy farms in the northwestern United States ([Hancock et al., 1998](#)). [LeJeune et al. \(2001b\)](#) found *E. coli* O157 in 1.3% of 473 water troughs at 98 different dairy cattle operations and one slaughter plant. [Van Donkersgoed et al. \(2001\)](#) found this organism in 12% of water troughs of feedlot cattle in southern Alberta, and noted a seasonal effect on its prevalence in the troughs. *E. coli* O157 was recovered from 25% of water samples collected at a beef cattle feedlot from May to August ([Sanderson et al., 2006](#)). Isolation of the same or similar molecular subtypes from both cattle and drinking water at the same farms or feedlots indicated a role for water in the transmission of *E. coli* O157:H7 among cattle ([Faith et al., 1996](#); [Shere et al., 1998](#); [Van Donkersgoed et al., 2001](#)). [Shere et al. \(2002\)](#) confirmed that drinking water contaminated with *E. coli* O157:H7 can disseminate this organism to cattle.

Water troughs may be contaminated with *E. coli* O157:H7 by cattle or their immediate environment, via feces, saliva, dust, feed, or bedding material (LeJeune *et al.*, 2001b; Shere *et al.*, 1998). Furthermore, several works have shown that this bacterium can persist for long periods of time in water. *E. coli* O157:H7 survived for at least 70 days in municipal drinking water at 5 °C, but was reduced to below enumerable levels after 42–49 days in the same water at 20 °C (Rice *et al.*, 1992). Similarly, Wang and Doyle (1998) showed that *E. coli* O157:H7 survived at least 91 days in municipal, reservoir, and lake waters at 8 °C, and that survival was greater at this lower temperature, in comparison to 15 and 25 °C. Rice and Johnson (2000) also reported persistence of this pathogen in cattle drinking water. LeJeune *et al.* (2001a) found that *E. coli* O157 survived at least 245 days in the sediments of microcosms simulating cattle water troughs, and was still infectious to calves after 183 days.

Transmission of *E. coli* O157:H7 by drinking water suggested that water may be a potential intervention target to control the pathogen in cattle production. LeJeune *et al.* (2004) saw no differences in *E. coli* O157:H7 prevalence in cattle feces or water trough sediments between pens of cattle supplied with chlorinated (1 ppm residual free chlorine) or non-chlorinated drinking water, likely as a result of large loads of organic matter in the troughs. Stevenson *et al.* (2004) concluded that electrolyzed oxidizing water may be useful for reducing this pathogen from livestock water, but the accumulation of organic material in the troughs (e.g., feces) would likely eliminate the bactericidal activity. Zhao *et al.* (2006) tested a large variety of chemical treatments, both alone and in combination, for the ability to inactivate *E. coli* O157:H7, O26:H11, and O111:NM in drinking water contaminated with cattle rumen contents or feces. Combinations of 0.1% lactic acid and 0.9% acidic calcium sulfate, with one of either 0.05% caprylic acid, 0.1% sodium benzoate, 0.5% butyric acid, or 100 ppm chlorine dioxide, were effective in inactivating enterohemorrhagic *E. coli* in water, but were not palatable to cattle. The addition of sodium caprylate (Amalaradjou *et al.*, 2006) and *trans*-cinnamaldehyde (Charles *et al.*, 2008) to cattle drinking water reduced *E. coli* O157:H7, but impacts on cattle water intake were not determined. Ozonation had little effect on inactivation of *E. coli* O157:H7 in water contaminated with rumen contents (Zhao *et al.*, 2006).

Though it has been shown that drinking water is commonly contaminated with *E. coli* O157:H7 and can transmit this pathogen to cattle, the impacts of measures to reduce *E. coli* O157:H7 in cattle drinking water on its prevalence in cattle have not been demonstrated. Smith *et al.* (2001) found that the prevalence of cattle in feedlot pens shedding *E. coli* O157:H7 was not correlated with the presence of the organism in the drinking water, or with the temperature, pH, or cleanliness of the water. Similarly, improved water trough hygiene did not reduce the risk of *E. coli* O157 in

young cattle (Ellis-Iversen *et al.*, 2008, 2009). Significant reduction of *E. coli* O157:H7 from cattle and the production environment as a result of drinking water decontamination must be demonstrated before investing in the development and implementation of water management programs for livestock producers (LeJeune and Wetzel, 2007).

D. Feces, manures, and soils

It is clear from the above discussion that transmission by either direct or indirect fecal–oral exposure is an important means of dissemination of *E. coli* O157:H7 in cattle. In this regard, cattle shedding *E. coli* O157:H7 provide the “fuel” for the maintenance of this pathogen in the environment and for the infection or reinfection of additional animals. Thus, feces, manure, and soils in the production environment are a significant source of transmission of this organism.

Horizontal transmission of *E. coli* O157:H7 among cattle was indicated by the work of Faith *et al.* (1996), who found that contact with areas previously occupied by cattle shedding the pathogen was an important factor in the spread of the organism in a dairy herd. Cobbold and Desmarchelier (2002) concluded that heavy fecal contamination of pen floors and hides was the primary source of STEC transmission to calves. The results of Bach *et al.* (2005a) suggested that feces on pen floors were a more likely source of *E. coli* O157:H7 to cattle than were contaminated feed or drinking water. Smith *et al.* (2001) observed that higher percentages of cattle in muddy feedlot pens shed *E. coli* O157:H7 than did cattle in pens in a normal condition, and reasoned that muddy pen soils may facilitate fecal–oral transmission. The importance of horizontal fecal–oral transmission of this pathogen is further suggested by research that has associated the presence of animals shedding high levels of *E. coli* O157:H7 with higher prevalence of fecal shedding and/or hide contamination in the pen or herd (Arthur *et al.*, 2009; Bach *et al.*, 2005a; Chase-Topping *et al.*, 2007; Cobbold *et al.*, 2007; Matthews *et al.*, 2006b; Stephens *et al.*, 2009). Many of these studies suggest that even very few cattle excreting high levels of the pathogen (“super-shedders”) are accountable for a large proportion of the total *E. coli* O157:H7 contamination of the pen and other cattle. Super-shedders of *E. coli* O157:H7 as effectors of the occurrence and levels of the pathogen in cattle production will be discussed further below.

Although the mammalian lower gastrointestinal tract is the primary habitat of *E. coli*, including *E. coli* O157:H7, this bacterium can survive for long periods of time in manure, feedlot surface material, and soils. When inoculated at initial levels of 10^5 CFU/g, *E. coli* O157:H7 could be recovered for up to 70 days from bovine feces stored at 5 °C (Wang *et al.*, 1996). Persistence was reduced at higher temperatures, but the pathogen

survived in bovine feces for up to 56 and 49 days at 22 and 37 °C, respectively, despite reductions in moisture content and water activity of the feces (Wang *et al.*, 1996). *E. coli* O157:H7 was recoverable by enrichment for 47 days from an aerated manure pile that was constructed of manure from cattle that were inoculated with the pathogen (Kudva *et al.*, 1998). Fukushima *et al.* (1999) reported that serotypes of STEC other than *E. coli* O157:H7 also can persist in bovine feces for several weeks. *E. coli* O157:H7 was shown to persist in feedlot surface material, and also to multiply in feedlot soils of permissible moisture and manure content (Berry and Miller, 2005). The importance of environmental persistence in the maintenance of *E. coli* O157:H7 in cattle production is further suggested by several studies that have reported that most isolates on a farm or feedlot are of one to a few genetic subtypes of this organism, which may predominate for months or years (Gannon *et al.*, 2002; Lahti *et al.*, 2003; LeJeune *et al.*, 2004; Shere *et al.*, 1998). In their longitudinal study of beef cattle at a commercial feedlot, LeJeune *et al.* (2004) found that most *E. coli* O157:H7 cattle isolates were one of four closely related genetic subtypes that persisted throughout the study, in spite of the large turnovers in cattle population. They concluded that the production environment as a reservoir may have a larger role as a source for transmission of the pathogen than do incoming cattle. Similarly, Lahti *et al.* (2003) observed persistent genetic subtypes of *E. coli* O157 in a Finnish cattle finishing unit, indicating that the finishing unit, rather than the introduction of new cattle, was the source of *E. coli* O157 at the farm.

The environmental persistence of this pathogen suggests a potential role for manure management or other similar interventions to reduce *E. coli* O157:H7 in cattle production, and is discussed further below. Managing manure to eliminate pathogens will reduce not only a source of *E. coli* O157:H7 for the reinfection of cattle, but also the risk of transmission of this organism to the environment, including water and human food and animal feed crops.

III. FACTORS AFFECTING THE PREVALENCE AND LEVELS OF *E. COLI* O157:H7 IN CATTLE AND THE PRODUCTION ENVIRONMENT

The development of control strategies to reduce *E. coli* O157:H7 will require the identification of biological, environmental, and/or management factors that affect its incidence in cattle and their production environments. Research investigations and epidemiological studies have identified a number of risk factors or management practices that can or may contribute to the occurrence of this pathogen, and that may be exploitable to reduce its numbers, persistence, and transmission in cattle.

A. Seasonality of shedding

Among those factors thought to affect the prevalence of *E. coli* O157:H7, only season has been repeatedly and most consistently demonstrated to influence the shedding of this pathogen by cattle (Barkocy-Gallagher *et al.*, 2003; Chapman *et al.*, 1997; Conedera *et al.*, 2001; Mechie *et al.*, 1997; Milnes *et al.*, 2009; Van Donkersgoed *et al.*, 1999). The prevalence of shedding of this pathogen typically increases during the warmer months, and is lowest in the winter. However, there are studies that have not observed this same seasonal pattern of shedding (Alam and Zurek, 2006; Ogden *et al.*, 2004; Sargeant *et al.*, 2000; Synge *et al.*, 2003). The housing of cattle during the winter in Scotland may account for the higher *E. coli* O157 prevalence observed during the winter months compared to summer months, perhaps by reducing exposure to the outside environment or by bringing the animals into closer proximity to one another (Ogden *et al.*, 2004; Synge *et al.*, 2003). Interestingly, although the prevalence of shedding the pathogen was higher during the cooler months when cattle were housed than during the warmer months when they were not, Ogden *et al.* (2004) observed that high-shedding cattle appeared to shed greater concentrations of *E. coli* O157 during the warmer months.

As a result of more favorable growth temperatures, the higher prevalence of *E. coli* O157:H7 in cattle during warmer seasons may be influenced by the ability to replicate in environmental reservoirs such as feed or water (Hancock *et al.*, 2001). Multiplication of the pathogen in cattle feeds has been demonstrated (Fenlon and Wilson, 2000; Lynn *et al.*, 1998). However, other studies have not observed *E. coli* O157:H7 growth in feeds, and indicate that there are a number of other factors that can influence the ability of this organism to survive or replicate in feeds, including feed medications, moisture content, organic acids, and pH (Bach *et al.*, 2002a; Van Donkersgoed *et al.*, 2001). Although *E. coli* O157:H7 has been shown to grow in experimental microcosms simulating cattle water troughs (Lejeune *et al.*, 2001b), cooler temperatures enhanced, rather than inhibited, the survival of the pathogen in water (Rice and Johnson, 2000; Rice *et al.*, 1992; Wang and Doyle, 1998). Similarly, numerous studies have shown that cooler temperatures can enhance the persistence of *E. coli*, including *E. coli* O157:H7, in manures and soils (Berry *et al.*, 2007; Ishii *et al.*, 2006; Kudva *et al.*, 1998; Topp *et al.*, 2003; Wang *et al.*, 1996). These observations make it interesting to speculate that greater persistence at cooler temperatures may be involved in the maintenance of this organism in the production environment during cooler winter weather when the rate of shedding by cattle typically is low.

Warmer temperatures, in comparison to very cold temperatures, may improve the transfer of *E. coli* O157:H7 in feedlot surface soils to and/or among cattle. Smith *et al.* (2001) found an association between the

environmental condition of a pen and the percentage of cattle shedding *E. coli* O157:H7, with higher prevalence of the organism occurring in cattle in muddy pens. Likewise, a USDA study in Nebraska examining the effects of diets with wet distillers grains with solubles (WDGS) observed that the percentages of *E. coli* O157:H7 either on hides or in feces of cattle in either treatment was lowest on a sampling day that followed a period of prolonged cold temperatures that kept pen surfaces frozen (Fig. 4.1; Wells *et al.*, 2009).

Cattle heat stress has been considered as a potential cause of the increased prevalence of the shedding of *E. coli* O157:H7 during the warmer seasons. However, clear effects of heat stress on the shedding of the pathogen by cattle have not been demonstrated (Brown-Brandl *et al.*, 2009; Edrington *et al.*, 2004; Fitzgerald *et al.*, 2003).

Factors other than temperature may influence the seasonal prevalence of *E. coli* O157:H7 in cattle. Edrington *et al.* (2006a) hypothesized that the seasonal variation of shedding is due to physiological responses of the animal as a result of changing day length. An experiment initiated in the early fall used artificial light in treatment pens to add 4–5 h/day to the daily number of hours of natural light, for a period of 60 days (Edrington *et al.*, 2006a). No differences in bovine fecal prevalence of *E. coli* O157:H7 were seen after 25 days of treatment, but at 53 days, prevalence was higher in the lighted treatment pens in comparison to the control pens, which received natural light only. Prevalence of the pathogen between the treatment and control cattle did not differ at 28 or 43 days following the removal of the light treatment. These results suggested a potential role for day length in the seasonal variation of *E. coli* O157:H7 in cattle. Follow-up work by these researchers has examined the effects of hormones that are known to respond to changes in day length, but results have been variable. Schultz *et al.* (2006) reported that melatonin had no effect on growth rates of *E. coli* O157:H7 in *in vitro* broth culture, and that there was no effect of exogenous melatonin on the *E. coli* O157:H7 fecal shedding patterns of sheep that were experimentally infected with the pathogen. In a separate study, there was no effect of a melatonin low-dose treatment of 0.5 mg/kg BW administered daily for 1 week, but when cattle were subsequently treated with a higher daily dose of 5.0 mg/kg BW, fecal prevalence of *E. coli* O157:H7 was lower in melatonin-treated cattle than in control cattle (Edrington *et al.*, 2008). There was no effect of tryptophan, a melatonin precursor, on fecal shedding of the pathogen (Edrington *et al.*, 2008). Melatonin treatment did not protect sheep from *E. coli* O157:H7 infection by horizontal transmission or alter fecal shedding of the organism after the sheep were colonized (Edrington *et al.*, 2009a). An involvement of the thyroid and thyroid hormones in seasonal fluctuations of *E. coli* O157:H7 shedding has also been suggested (Edrington

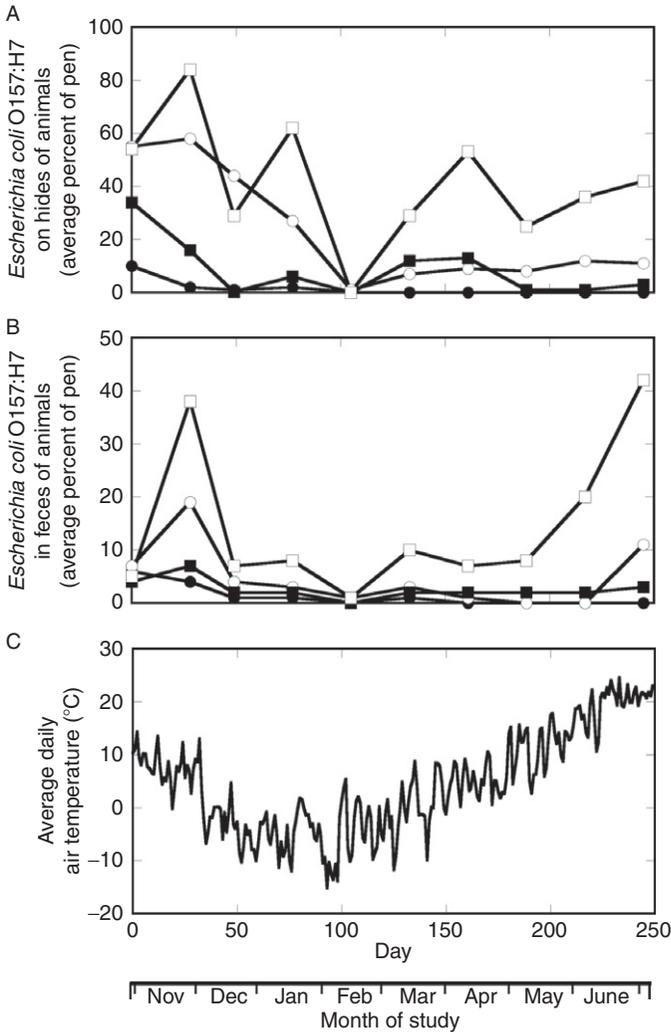


FIGURE 4.1 Percentage of samples positive (open symbols) or enumerable (closed symbols) for *Escherichia coli* O157:H7 on hides (A), in feces (B), and on daily average temperature (C) over time of the study. Control diet (CON) pen groups are represented by circles (○,●) and wet distillers grains with solubles diet (WDGS) pen groups are represented by squares (□,■). The growing phase comprises days 1–77, and the finishing phase comprises day 78–245 of production. Each month of the study is denoted in the bar across the bottom of the figure. Reprinted from Wells *et al.* (2009).

et al., 2007; Schultz *et al.*, 2005). Further work is needed to confirm the impacts of day length and day length-responsive hormones on *E. coli* O157:H7 in cattle.

As noted earlier, fly populations increase during the warmer seasons, and flies have been demonstrated to transmit *E. coli* O157:H7 to cattle (Ahmad *et al.*, 2007). However, any influence of flies on the prevalence of the pathogen in cattle or as a cause of seasonal shedding has not been shown.

Human foodborne disease caused by *E. coli* O157:H7 exhibits a seasonal pattern of occurrence that parallels the seasonal increased shedding of the pathogen by cattle (Rangel *et al.*, 2005; Slutsker *et al.*, 1997; Waters *et al.*, 1994). While all or none of these above factors may contribute to the seasonality of shedding of this pathogen, the regularity of this phenomenon suggests that the identification and confirmation of its cause(s) may point to strategies to reduce shedding by cattle, thereby reducing the risk of human infection.

B. High-level shedders of *E. coli* O157:H7

Recent work has highlighted the impact of high-level shedders of *E. coli* O157:H7 on the prevalence and transmission of this pathogen. Most cattle shed *E. coli* O157:H7 in feces at concentrations that are below the detectable levels of enumeration procedures, but levels as high as 10^5 – 10^6 CFU/g of feces have been reported (Brichta-Harhay *et al.*, 2007; Omisakin *et al.*, 2003; Robinson *et al.*, 2004). Cattle that shed high numbers of *E. coli* O157:H7 ($\geq 10^3$ – 10^4 CFU/g of feces) are called “super-shedders,” and this small proportion of super-shedding cattle is responsible for a large proportion of *E. coli* O157:H7 contamination in a production environment, which may in turn drive the *E. coli* O157:H7 prevalence of cattle in that environment (Bach *et al.*, 2005a; Chase-Topping *et al.*, 2007, 2008; Cobbold *et al.*, 2007; Low *et al.*, 2005; Matthews *et al.*, 2006a; Stephens *et al.*, 2009).

The significance of super-shedders of *E. coli* O157:H7 in the dissemination and maintenance of this pathogen in cattle production has been substantiated by a number of studies. Bach *et al.* (2005a) noted that persistent and high-level shedding by individual animals were likely the source of infection for other animals in the pen. Similarly, the presence of high-level shedders of *E. coli* O157:H7 in feedlot pens was associated with higher prevalence of the pathogen among cattle in the same pen, while cattle that were negative for the pathogen were more likely to have been in a pen that did not have a super-shedding animal (Cobbold *et al.*, 2007). High-level rectal carriage of *E. coli* O157 in cattle at slaughter was associated with a higher risk for *E. coli* O157 fecal-positive animals in the same lot (Low *et al.*, 2005). The presence of a high-level shedder of *E. coli* O157:H7 on a farm was associated with higher prevalence of the pathogen among cattle on that farm (Chase-Topping *et al.*, 2007). Mathematical modeling of prevalence and transmission dynamics of *E. coli* O157 on cattle farms in Scotland indicates that the distribution of prevalence is best

explained when a small proportion of cattle (the super-shedders) are responsible for most of the infections in the rest of the population, and that 80% of the *E. coli* O157 infections arise from the 20% of cattle that are shedding high levels of the pathogen (Matthews *et al.*, 2006a,b).

High-level fecal shedding of *E. coli* O157:H7 has also been linked to increased hide contamination, which is an important source of this pathogen on beef carcasses at harvest (Arthur *et al.*, 2007, 2009; Stephens *et al.*, 2009). Arthur *et al.* (2009) suggested that cattle hide contamination could be minimized if fecal concentrations of *E. coli* O157:H7 were reduced below 200 CFU/g. In a study examining feedlot cattle, Stephens *et al.* (2009) observed a larger impact of high-level shedders of *E. coli* O157:H7 on hide prevalence than fecal prevalence of penmates. Additionally, Fox *et al.* (2008b) recently reported that the probability of carcass contamination with *E. coli* O157 was significantly associated with the presence of a high-shedding animal within the same truckload of cattle. The association of high-shedding cattle with carcass contamination extends the chain of events linking super-shedding cattle and human disease risk outlined in Fig. 4.2 by Chase-Topping *et al.* (2008) to include foodborne exposure in addition to environmental exposure.

Factors or mechanisms that result in high-level shedding of *E. coli* O157:H7 by cattle are not fully understood. However, high levels of fecal excretion and longer duration of shedding are associated with colonization at the terminal rectum (Cobbold *et al.*, 2007; Davis *et al.*, 2006; Lim *et al.*, 2007; Low *et al.*, 2005; Naylor *et al.*, 2003). *E. coli* O157 from high-level shedding cattle in Scotland were more likely to be phage type 21/28 than isolates from low-shedding cattle, identifying a potential pathogen-associated risk factor (Chase-Topping *et al.*, 2007). Further investigation is needed to determine host, microbial, or other environmental factors that influence high-level shedding of *E. coli* O157:H7; identification of these factors may indicate strategies to reduce this occurrence in cattle.

That even very few animals shedding high levels of the pathogen can be responsible for a large proportion of the total *E. coli* O157:H7 contamination of the pen and other cattle suggests targeting these animals may be an approach to substantially reduce the risk for human illness caused by this pathogen. Potential strategies include detection, removal, and/or application of intervening treatments to high-level shedders before introduction to the herd or before slaughter (Chase-Topping *et al.*, 2008; Cobbold *et al.*, 2007; Davis *et al.*, 2006; Matthews *et al.*, 2006a,b; Naylor *et al.*, 2003, 2007). As colonization of the terminal rectal mucosa is associated with high-level fecal shedding of this pathogen, recent studies have targeted this site to reduce *E. coli* O157:H7 in cattle. Direct application of polymyxin B or chlorhexidine to the rectal mucosa of experimentally colonized calves reduced *E. coli* O157:H7 concentrations in feces (Naylor *et al.*, 2007). Similarly, a combination of bacteriophage application to the

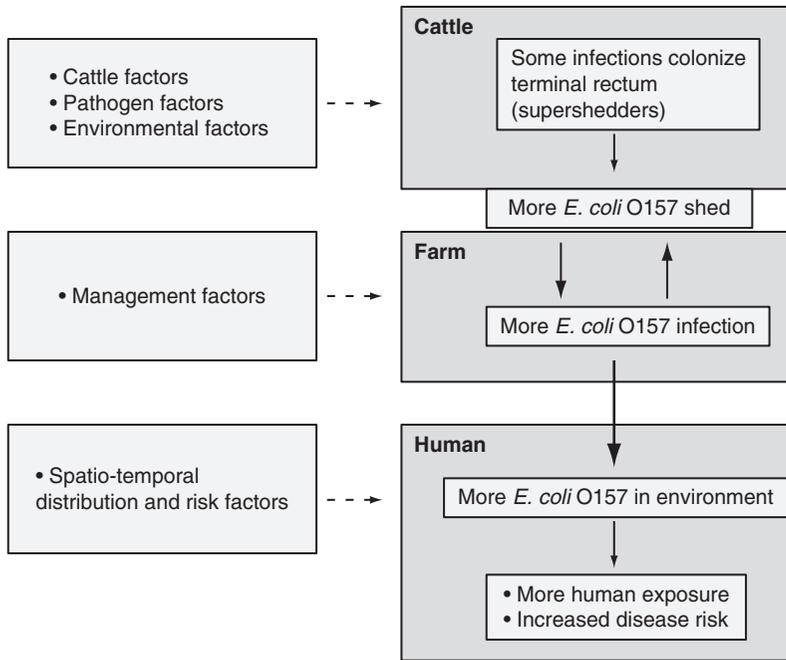


FIGURE 4.2 The chain of events that link super-shedding cattle and the risk of human infection of *Escherichia coli* O157. Vertical arrows represent the epidemiological processes involved, with larger arrows indicating an increased risk. Dotted arrows indicate factors that influence risk. Adapted by permission from Macmillan Publishers Ltd: Chase-Topping *et al.* (2008), copyright 2008.

rectoanal junction and oral administration of bacteriophage in drinking water reduced *E. coli* O157:H7 shedding by cattle, although it did not eliminate the pathogen (Sheng *et al.*, 2006). In a separate study, oral administration of bacteriophage was determined to be more effective than rectal administration for reducing *E. coli* O157:H7 shedding (Rozema *et al.*, 2009).

Chase-Topping *et al.* (2008) have provided a recent comprehensive review that discusses the implications of super-shedders of *E. coli* O157:H7 on the transmission dynamics of this pathogen in cattle, the risk of human illness, and the prospects for disease control.

C. Diet effects on shedding and persistence

The ruminant animal differs significantly from monogastric animals, such as humans, rodents, and swine, in gastrointestinal tract physiology and digestion. In particular, ruminant animals have a rumen, or pregastric

compartment of the stomach, in which symbiotic microbial fermentation occurs and volatile fatty acids are absorbed (Wells and Varel, 2005). As a consequence, ruminant animals are adept at digesting and utilizing complex forages; however, many modern cattle feeding operations primarily feed high-energy, high-starch corn-based diets. Arguments relative to how cattle are fed and the impact on *E. coli* O157:H7 have been extensively debated, and recent reviews have detailed the results of animal diet studies on *E. coli* O157:H7 shedding (Callaway *et al.*, 2009; Jacob *et al.*, 2009a).

Early work suggested that cattle fed hay would have lower *E. coli* O157:H7 in feces than cattle fed corn (Diez-Gonzalez *et al.*, 1998). This hypothesis was derived from observations that hay-fed animals had much fewer fecal *E. coli* and acid-resistant *E. coli* than animals fed concentrate (high energy) diets, and acid resistance was emphasized. Nutrient utilization and growth and colonization capabilities may be better indicators of fitness for a niche, and in the case of *E. coli* O157:H7 in feces, the numbers of generic *E. coli* more so than acid-resistant *E. coli* may be a better indicator (Berry *et al.*, 2004, 2006). Commensal strains of *E. coli* and virulent strains of *E. coli* O157:H7 differed in their abilities to utilize different carbon sources, and in general, commensal *E. coli* oxidized more substrates than did *E. coli* O157:H7 (Durso *et al.*, 2004). However, commensal *E. coli* and *E. coli* O157:H7 had similar fitness for growth and were similar with regard to physiological parameters associated with colonization of the gastrointestinal tract (Durso *et al.*, 2004; Jacobsen *et al.*, 2009).

Steers fed hay or corn silage had similar levels of generic *E. coli* in ruminal fluid and feces, but generic *E. coli* levels in ruminal fluid and feces increased for both diets when the same animals were converted to a corn diet (Berry *et al.*, 2006). However, no effects of diet on the prevalence of *E. coli* O157:H7 in feces were noted. Interestingly, *E. coli* O157:H7 were rarely found in samples from the rumen over the 9-month duration of the study and the levels of generic *E. coli* were 100- to 1000-fold lower in the ruminal fluid than the feces, indicating that the rumen is not a likely reservoir for this pathogen. Likewise, in animals inoculated with *E. coli* O157:H7, Buchko *et al.* (2000a) noted a rapid elimination of the pathogen from the rumen, and Van Baale *et al.* (2004) did not recover *E. coli* O157:H7 from rumen tissues collected 11 weeks after experimental inoculation. The potential effects of bovine diet on *E. coli* O157:H7 are most likely to be due to changes in the lower gastrointestinal tract where *E. coli* is better adapted and the niche is expanded.

In numerous studies, the potential effects of diet have often been evaluated using cattle experimentally inoculated with *E. coli* O157:H7 (Jacob *et al.*, 2009a). These studies by design are super-physiological and the pathogen dose typically is in far excess of what the animal would

ingest in normal transmission; however, they do provide some insight into colonization and persistent shedding of the pathogen. In comparison, studies examining shedding in naturally colonized animals are biologically more relevant to the feedlot system, but often dietary effects are not evident unless a large number of animals have been sampled repeatedly. Needless to say, the potential effects of diet on *E. coli* O157:H7 shedding should be surmised from a large body of work more so than any single study.

Cattle in feedlots are fed energy-dense grain rations to improve growth and produce a high quality product, and in the United States and Canada most feedlot diets are based on grains such as corn or barley. In order to be implemented in a feedlot, any pathogen reduction benefit from a diet should not come at an increased cost in production. Barley is a grain that is grown and fed in Canada and the U.S. Northern Plains region, and in animals experimentally inoculated (Buchko *et al.*, 2000a) and naturally infected (Berg *et al.*, 2004), fecal prevalence was higher for cattle fed barley compared to cattle fed cracked (dry-rolled) corn. Barley has a starch content that is rapidly digested in the rumen, which results in less starch entering the lower digestive tract. Buchko *et al.* (2000a) observed lower pH in feces of animals fed cracked corn than those fed barley, and hypothesized that lower prevalence seen with corn could be due to a less hospitable fecal environment. Processing of grain to increase surface area can result in a grain that is more digestible in the rumen and passes less starch to the lower intestine, and processing such as steam-flaking are common practices in the U.S. Southern Plains region where many feedlots are concentrated. In a study by Fox *et al.* (2007b), steam-flaked grains (sorghum or wheat) were associated with higher fecal prevalence for *E. coli* O157:H7 compared to dry-rolled equivalents. When steam-flaked corn was fed to naturally infected cattle sampled over time, fecal *E. coli* O157 prevalence was only slightly higher compared to the dry-rolled corn, and steam-flaking did result in less fecal starch and higher fecal pH (Depenbusch *et al.*, 2008). However, in this latter study, neither fecal starch amount or fecal pH was associated with presence or absence of the pathogen. As a whole, fecal shedding in feedlot cattle may be manipulated by processing of the energy-rich grains fed to cattle, but the mechanism(s) to date to fully explain this phenomenon has remained elusive.

In recent years, grains typically fed to cattle have been shifted to the production of ethanol and cattle have been fed the coproducts, collectively known as distillers grains. Corn, in particular, has been a grain most often used for ethanol production and its coproduct is fed either as wet distillers grain, typically with solubles (WDGS), or to a lesser extent, as dried distillers grain with or without solubles (DDGS or DDG). Brewers' grains are coproduct residues of beer production fed to cattle, and an

epidemiological study identified this animal feed as a factor associated with sixfold increased odds of detecting *E. coli* O157 in fecal samples (Dewell *et al.*, 2005). A preliminary study feeding WDGS at six different levels from 0% to 50% of the diet (dry matter basis) reported no differences in probability for *E. coli* O157:H7 in feces, but did report differences in the probability for detecting *E. coli* O157:H7 in terminal rectum tissues of cattle fed different levels of WDGS (Peterson *et al.*, 2007b). Experiments with inoculated animals demonstrated that cattle fed 25% DDG diets consistently shed nearly 2-log higher levels of *E. coli* O157:H7 over the last 7 days of the 42-day study, and at necropsy, significant numbers of the pathogen were found associated with tissue samples collected throughout the intestinal tract (Jacob *et al.*, 2008c). Additional work by this laboratory observed that feeding 25% DDG (on dry matter basis) to naturally infected cattle more than doubled the prevalence of *E. coli* O157 in fecal pat samples collected from pen floors over a 12-week study period (Jacob *et al.*, 2008a), and also found that feeding 25% WDGS (on dry matter basis) was associated with increased prevalence at one sample date but there was no difference on a second sample date (Jacob *et al.*, 2008b). A separate study did not observe an association of feeding DDGS and *E. coli* O157:H7 fecal prevalence in steam-flaked corn-based diets (Jacob *et al.*, 2009b).

A recent large feedlot study following the same animals for more than 9 months through growing and finishing phases of production observed a significant increase in fecal prevalence for growing animals fed 14% WDGS (dry matter basis) diet compared to a 0% WDGS (Wells *et al.*, 2009). During the finishing phase, the steers fed 40% WDGS (dry matter basis) diet had higher fecal prevalence for *E. coli* O157:H7 and a higher percentage of the animals were found to be shedding the pathogen at enumerable levels in the feces. As observed for processed grains fed to cattle, diets high in distillers grains would result in less starch passing to the hindgut and, as a consequence, higher fecal pH compared to a corn diet (dry-rolled or high moisture; Wells *et al.*, 2009). In addition, the diet high in WDGS had lower levels of lactate and total volatile fatty acids in the feces, supporting the hypothesis that WDGS diets could alter the gastrointestinal tract environment and promote a more hospitable environment to support pathogen growth and persistence in the intestine. Cattle fed WDGS had at least twofold higher levels of generic *E. coli* in the feces compared to cattle fed corn, indicating that the microbial niche may have been expanded due to diet, in addition to diet providing a more favorable environment. How diets with distillers grains may result in an expanded niche for *E. coli* and higher prevalence for *E. coli* O157:H7 has yet to be determined, but could be as simple as a component within the distillers grain product or as complex as an alteration in gut chemistry. For example, Fox *et al.* (2009b) conducted *in vitro* studies with fecal

suspensions and noted that mucus constituents stimulated *E. coli* O157:H7 growth, and as such, diets that alter mucin production in the intestine may alter levels and persistence of the pathogen.

Studies examining the effects of cattle diet on *E. coli* O157:H7 have primarily focused on the gastrointestinal tract and fecal incidence. As a result, the interaction of diet \times environment on the levels and persistence of *E. coli* O157:H7 in cattle production has not been fully appreciated. In addition to influencing the numbers of this pathogen that are shed in the feces, cattle diets can impact the extent to which *E. coli* O157:H7 can persist in manure or feedlot surface soils. Cattle diets that result in enhanced survival in manure and the production environment can further contribute to the scenarios depicted in Fig. 4.2, by (1) increasing the opportunities for fecal–oral exposures resulting in additional or repeated *E. coli* O157:H7 cattle infections, and/or (2) increasing the risks for further environmental contamination (e.g., water, soils, food and feed crops), that in turn increase the risk for human exposure and infection.

Diets with lower levels of corn increased the persistence of *E. coli* O157:H7 in bovine feces and manure (Varel *et al.*, 2008, 2010; Wells *et al.*, 2005). At $-10\text{ }^{\circ}\text{C}$, *E. coli* O157:H7 survived longer in feces from barley-fed cattle compared to corn-fed cattle, and at $22\text{ }^{\circ}\text{C}$, it grew to higher numbers in feces from barley-fed cattle compared to corn-fed cattle (Bach *et al.*, 2005b). In diets with dry-rolled corn, both naturally occurring generic *E. coli* and/or inoculated *E. coli* O157:H7 persisted in greater numbers longer in manure from cattle fed 20% and 40% WDGS, than in manure from cattle fed 0% WDGS and a higher percentage of dry-rolled corn (Varel *et al.*, 2008, 2010). The extended persistence of *E. coli* O157:H7 in manure from cattle fed distillers grains may be responsible in part for the higher prevalence of this pathogen on hides and feces of cattle that has been reported for this feedstuff (Jacob *et al.*, 2008a,b; Wells *et al.*, 2009). Indeed, diet effects on the persistence of this pathogen and the subsequent effects on *E. coli* O157:H7 prevalence may account for some of the inconsistencies regarding the effects of particular dietary components on the prevalence of this pathogen in cattle (e.g., grain vs. forage, grain type and processing, distillers grains; for a recent review, see Jacob *et al.*, 2009a). Thus, studies examining the impact of cattle diets should consider not only the effect of the diet on the prevalence and numbers of *E. coli* O157:H7 shed in feces, but also the effect of that diet on the numbers and persistence of *E. coli* O157:H7 in the manure.

D. Animal stress

It is commonly assumed that animal stress has a detrimental effect on microbial food safety risk; however, direct cause-and-effect relationships between animal stress responses and increased carriage and shedding of

human pathogens have not been clearly demonstrated (Rostagno, 2009). In addition, it is generally accepted that animal stress can suppress or alter the immune response, leading to increased susceptibility to infection (Rostagno, 2009; Salak-Johnson and McGlone, 2007). However, *E. coli* O157:H7 commonly occurs in cattle without apparent negative effects on health or performance and is not generally considered to be a pathogen of cattle (Berry *et al.*, 2006; Cray and Moon, 1995). Nonetheless, *in vitro* work has indicated that neuroendocrine hormones and other immune-modulating molecules associated with stress responses may influence the growth of *E. coli*, including *E. coli* O157:H7, and its ability to colonize a host, which is likely a reflection of its status as a commensal. The catecholamine norepinephrine is released into the gastrointestinal tract upon animal stress (Rostagno, 2009). *E. coli* has been demonstrated to take up norepinephrine and to respond to norepinephrine with stimulated growth and expression of molecules associated with virulence and colonization, including adhesin, Shiga-like toxins, and LEE-encoded proteins (Bansal *et al.*, 2007; Freestone *et al.*, 2007, 2008; Kinney *et al.*, 2000; Lyte *et al.*, 1997; Sperandio *et al.*, 2003). Norepinephrine enhanced adherence of *E. coli* O157:H7 to the intestinal mucosa in a bovine ligated ileal loop model of infection (Vlisidou *et al.*, 2004). The glucocorticoid dexamethasone induces immunosuppression in cattle, and has been used to enhance the susceptibility of cattle to a variety of bacterial, viral, and protozoal diseases for research purposes, including *E. coli* O157:H7 infections (Dean-Nystrom *et al.*, 2008; Sreerama *et al.*, 2008; Stoffregen *et al.*, 2004). Dexamethasone treatment enhanced the susceptibility of weaned calves to *E. coli* O157:H7 colonization, and both fecal and intestinal levels of the pathogen were higher in dexamethasone-treated calves than in nontreated calves (Dean-Nystrom *et al.*, 2008).

In comparison to these laboratory studies, results of *in vivo* work investigating the practical implications of animal stress on the prevalence and shedding of *E. coli* O157:H7 have been generally less definitive, although a number of potential stressors have been identified. Abrupt weaning was associated with a higher prevalence of *E. coli* O157 in calves (Herriott *et al.*, 1998). Similarly, cattle weaning and movement stress were risk factors for the presence of a high-level shedder of *E. coli* O157 on a farm (Chase-Topping *et al.*, 2007). Bach *et al.* (2004) reported that long-haul transport and a lack of preconditioning increased fecal shedding of *E. coli* O157:H7 in range calves, and concluded that the stresses of weaning, transport, and relocation likely increased their susceptibility to infection. Conversely, Schuehle Pfeiffer *et al.* (2009) did not observe an increase in fecal shedding of *E. coli* O157:H7 by feedlot cattle after transportation to a processing facility. The fasting that is associated with the transportation may both make animals more susceptible to *E. coli* O157:H7 colonization and increase their shedding of the pathogen (Buchko *et al.*, 2000b; Cray

et al., 1998; Kudva *et al.*, 1995), although not all studies have seen these effects (Harmon *et al.*, 1999). Thus, fasting may be responsible in part for the effects of transport on the shedding of *E. coli* O157:H7 (Callaway *et al.*, 2009). Transportation to and lairage at beef processing facilities has been associated with increased prevalence of *E. coli* O157:H7, but this increase may be due to exposure and contact to contaminated fecal material present in the transport truck and lairage environments (Arthur *et al.*, 2007, 2008; Dewell *et al.*, 2008; Fox *et al.*, 2008b), rather than, or in addition to, increased fecal shedding (Bach *et al.*, 2004).

Brown-Brandl *et al.* (2009) did not observe relationships between handling stress of feedlot cattle, as measured by temperament score, and either *E. coli* O157:H7 prevalence or concentrations or generic *E. coli* concentrations in their feces. Schuehle Pfeiffer *et al.* (2009) reported that cattle with calmer temperaments, as compared to cattle with excitable temperaments, had higher fecal prevalence of this pathogen, which is contrary to the typical hypotheses regarding the effects of animal stress on pathogen carriage and shedding.

Edrington *et al.* (2004) did not observe a clear effect of heat stress on the shedding of *E. coli* O157:H7 or *Salmonella* in lactating dairy cattle. Similarly, Brown-Brandl *et al.* (2009) did not find clear trends between the heat stress levels experienced by individual beef heifers with either fecal generic *E. coli* concentrations or *E. coli* O157:H7 concentrations or prevalence.

As recently reviewed by Rostagno (2009) and Salak-Johnson and McGlone (2007), the relationships between stressors, immunity, and pathogen infection in livestock are complex. Many factors may influence livestock response to stress or the results of studies examining these responses, including stressor type, duration of stress (acute vs. chronic), sample type, or the time of sample collection relative to the onset of stress (Salak-Johnson and McGlone, 2007). Moreover, confounding factors include the methodology of stress assessment or the lack of specificity of the selected parameter to measure, as well as the variability in responses of individual animals to challenges or stressors (Rostagno, 2009). Further research with solid experimental designs and appropriate controls is needed to clarify the relationships between animal stress responses and food safety risk.

E. Other effectors

As noted above, norepinephrine and also epinephrine can increase virulence gene expression and colonization by *E. coli* O157:H7 *in vitro* (Bansal *et al.*, 2007; Freestone *et al.*, 2007, 2008; Kinney *et al.*, 2000; Lyte *et al.*, 1997; Sperandio *et al.*, 2003). Beta-agonists are synthetic homologues to

norepinephrine and epinephrine, and have been approved for dietary use in feedlot cattle to improve lean muscle growth. Increased fecal shedding of *E. coli* O157:H7 was observed with inoculated lambs treated with beta-agonists (Edrington *et al.*, 2006c), but in feedlot studies with cattle, no significant increase in fecal prevalence for *E. coli* O157:H7 was observed (Edrington *et al.*, 2006b, 2009b).

Feedlot cattle have been fed ionophores for decades to alter rumen microbial flora and to improve feed conversions (Russell and Houlihan, 2003), and an early study observed a tendency for increased *E. coli* O157 herd prevalence when ionophores were fed (Herriott *et al.*, 1998). Gram-positive bacteria are more sensitive to ionophores (Russell and Houlihan, 2003), and commonly fed ionophores, such as monensin or lasalocid, have little effect on the Gram-negative *E. coli* O157:H7 in pure culture studies (Bach *et al.*, 2002b; Edrington *et al.*, 2003). In feedlot studies with cattle fed grain diets, neither monensin or tylosin altered *E. coli* O157:H7 fecal prevalence (Jacob *et al.*, 2008b; McAllister *et al.*, 2006). In experimentally inoculated steers, *E. coli* O157:H7 levels in feces from animals fed grain were not different when also fed monensin, but in feces from animals fed forage, the duration of shedding enumerable levels was shorter when monensin was added to the diet (Van Baale *et al.*, 2004). Potential diet and ionophore interactions have not been fully studied, but *in vitro* incubations with inoculated rumen fluid had lower levels of *E. coli* O157:H7 when monensin and tylosin were added to rumen fluid from cattle fed forage than in rumen fluid from cattle fed grain (McAllister *et al.*, 2006).

IV. PREHARVEST CONTROL OF *E. COLI* O157:H7

Because of the linkage of *E. coli* O157:H7 fecal shedding and hide prevalence to beef carcass contamination, intervention strategies targeted at the live animal are anticipated to reduce the risk of human illness associated with bovine food products. In addition, the application of effective preharvest control measures that reduce *E. coli* O157:H7 in the live animal should not only reduce the prevalence of this organism in beef and milk, but also reduce the incidence of environmental contamination by this organism via cattle waste, thereby further reducing the risk of human foodborne and waterborne illness. Some preharvest control procedures currently are in use or are available for application, while some procedures will require regulatory approval and/or additional research to determine their effectiveness for reducing *E. coli* O157:H7 in cattle.

A. Vaccines

Cattle producers may readily adopt an effective vaccine against *E. coli* O157:H7 because they are already familiar with vaccine use and can easily incorporate a vaccine into existing cattle management systems (Loneragan and Brashears, 2005).

Considerable work has examined the ability of a vaccine against *E. coli* O157:H7 type III secreted proteins (Bioniche Life Sciences, Inc., Belleville, Ontario, Canada) to reduce *E. coli* O157:H7 in cattle. Type III secreted proteins are critical for *E. coli* O157:H7 intestinal colonization of cattle, indicating their utility as a vaccine target (Naylor *et al.*, 2005). In early work, vaccination with type III secreted proteins reduced the prevalence, duration, and magnitude of *E. coli* O157:H7 fecal shedding in experimentally inoculated cattle, and also reduced the fecal prevalence of the pathogen in naturally colonized cattle (Potter *et al.*, 2004). However, the vaccine did not significantly reduce pen prevalence of fecal *E. coli* O157:H7 in feedlot cattle in a large field trial (Van Donkersgoed *et al.*, 2005). Following reformulation of the vaccine, several studies have shown efficacy of this vaccine to reduce, though not eliminate, *E. coli* O157:H7 in cattle. Cattle receiving the vaccine were less likely to be colonized at the terminal rectum (Peterson *et al.*, 2007b; Smith *et al.*, 2009b) and less likely to shed *E. coli* O157:H7 in feces (Moxley *et al.*, 2009; Peterson *et al.*, 2007c; Smith *et al.*, 2009a). A two-dose regimen of the vaccine reduced *E. coli* O157:H7 colonization of the terminal rectum, from 17.0% of nonvaccinated cattle to 2.9% of vaccinated cattle (Smith *et al.*, 2009b). Smith *et al.* (2009a) tested the effect of vaccinating all of the cattle within a region of a feedlot (regional vaccination) on *E. coli* O157:H7 rectal colonization, fecal shedding, and hide contamination, and observed that regional vaccination reduced the probability for cattle to have *E. coli* O157:H7-positive hides, as a consequence of reduced environmental *E. coli* O157:H7. This commercial vaccine is fully licensed for use in Canada and Bioniche Life Sciences, Inc. is working to meet the requirement for a U.S. conditional license (Bioniche Life Sciences, Inc., 2008).

A vaccine targeting siderophore receptor and porin proteins (Epitopix, LLC, Wilmar, MN) recently received a conditional license for use in cattle in the United States. (Epitopix, LLC, 2009). The vaccine tended to reduce *E. coli* O157:H7 fecal prevalence and fecal concentrations of the pathogen in *E. coli* O157:H7-inoculated calves (Thornton *et al.*, 2009). In two large feedlot trials examining two- and three-dose vaccine regimens, Thomson *et al.* (2009) observed measures of efficacy for both dose regimens, but saw evidence for a greater efficacy for three doses. Vaccination with three doses of the vaccine was associated with a two log reduction of *E. coli* O157 in feces (Thomson *et al.*, 2009). Subsequent work in naturally colonized feedlot cattle compared the use of 2- and 3-ml doses of the vaccine

administered in two-dose regimens, and found that the 3-ml dose effectively reduced the prevalence of *E. coli* O157, the number of days that animals tested positive, and the number of days that animals were identified as high-shedders of the pathogen ($> 10^3$ CFU/g of feces), compared to the placebo (Fox *et al.*, 2009a).

Other experimental vaccines have potential to reduce *E. coli* O157:H7 in cattle. Immunization of the sows using a vaccine containing *E. coli* O157:H7 intimin protected the suckling piglets from *E. coli* O157:H7 infection and intestinal damage (Dean-Nystrom *et al.*, 2002). Vaccination of experimentally infected calves with a combination of type III secreted proteins (EspA, intimin, and Tir) reduced *E. coli* O157:H7 concentrations in feces and the total load of the pathogen that was shed (McNeilly *et al.*, 2010). In contrast, vaccination of calves with EspA alone induced an immune response, but did not protect against *E. coli* O157:H7 intestinal colonization (Dziva *et al.*, 2007). Intramuscular immunization with H7 flagellin reduced colonization rates and delayed peak shedding of *E. coli* O157:H7 in orally inoculated calves, but did not reduce total pathogen shedding (McNeilly *et al.*, 2008). A preliminary study indicated that an experimental bacterin vaccine could reduce *E. coli* O157 prevalence in feces and on hides, but statistical validation is needed (Woerner *et al.*, 2006b).

B. Probiotics or direct-fed microbials

The microbial flora is an important component of the gastrointestinal tract, and certain bacteria have long been recognized for beneficial properties and good health (Wells and Varel, 2005). Mechanistically, beneficial bacteria can prevent harmful bacterial colonization by competitively excluding, producing antibacterial compounds, and/or promoting healthy immune function. Probiotics or direct-fed microbials are live bacteria fed to a host to elicit a beneficial response, and are typically, but not limited to, *Lactobacillus* spp. strains. In cattle, numerous probiotics have been identified and tested for efficacy against *E. coli* O157:H7 in cattle (as reviewed by Callaway *et al.*, 2009; Loneragan and Brashears, 2005; Oliver *et al.*, 2009; Sargeant *et al.*, 2007). Some effective probiotics reviewed previously include, either individually or in combinations, *Enterococcus* (*Streptococcus*) *faecium*, *L. acidophilus*, *L. casei*, *L. fermentum*, *L. gallinarum*, *L. plantarum*, *Propionibacterium freundenreichii*, and *Streptococcus bovis*, and these bacterial types interestingly are localized in the rumen or small intestine. As a direct-fed microbial, selected *L. acidophilus* strains, alone or in combination with *P. freundenreichii*, have been the most thoroughly studied and often are very effective at reducing the prevalence of fecal shedding of *E. coli* O157:H7 when dosed at 10^9 cells per animal daily (Peterson *et al.*, 2007a; Tabe *et al.*, 2008; Younts-Dahl *et al.*, 2005).

A reduction in *E. coli* O157 fecal concentrations has been reported in one study where fecal positive samples were subsequently analyzed using a most probable number procedure in combination with immunomagnetic separation (Stephens *et al.*, 2007a). Feeding *Lactobacillus*-based direct-fed microbials also have been shown to reduce the prevalence of *E. coli* O157:H7 on cattle hides (Brashears *et al.*, 2003; Stephens *et al.*, 2007b). Commensal *E. coli*, including colicinogenic strains, have also been tested for probiotic potential against *E. coli* O157:H7 in inoculated calves, but results have been limited to date (Schamberger *et al.*, 2004; Tkalcic *et al.*, 2003). Laboratory studies indicated that *E. coli* O157:H7 can become resistant to individual colicins, so effective treatments may require a cocktail of strains producing different colicin types (Schamberger and Diez-Gonzalez, 2005). Recent work using *Bacillus subtilis* as a direct-fed microbial in cattle did not effect prevalence or levels of *E. coli* O157:H7 in feces or on hides (Arthur *et al.*, 2010).

C. Bacteriophage

Bacteriophages are viruses of bacteria, and their host specificity and ability to destroy their host bacteria (in the case of virulent, or lytic, bacteriophages) in the process of amplifying their own numbers has made bacteriophages attractive candidates for reducing bacterial pathogens in foods and food animals (Greer, 2005; Johnson *et al.*, 2008). Bacteriophages are obligate parasites, and as a consequence, share a common ecology with their bacterial hosts. Bacteriophages that infect *E. coli* O157:H7 have been found throughout the feedlot environment, including cattle feces, manure slurries, and water troughs (Callaway *et al.*, 2006; Niu *et al.*, 2009b; Oot *et al.*, 2007). Observations of fluctuations in the prevalences of *E. coli* O157:H7-infecting bacteriophages and *E. coli* O157:H7, negative correlations between bacteriophage and *E. coli* O157:H7, and low frequencies of fecal samples containing both *E. coli* O157:H7-infecting bacteriophages and their host suggest a classical predator–prey relationship between *E. coli* O157:H7 and its bacteriophages, and indicate the role that bacteriophages may play in the ecology of this pathogen (Callaway *et al.*, 2006; Niu *et al.*, 2009b; Oot *et al.*, 2007).

Several *in vitro* studies have demonstrated the successful elimination of *E. coli* O157:H7 with bacteriophages (Bach *et al.*, 2003a; Kudva *et al.*, 1999; Raya *et al.*, 2006). The effectiveness of bacteriophage treatments to reduce this pathogen in live animals has been more variable, and elimination of *E. coli* O157:H7 has not been demonstrated. Although bacteriophage DC22 eliminated *E. coli* O157:H7 in an artificial rumen system, it did not reduce the levels of the pathogen shed by inoculated lambs when given in a single dose (Bach *et al.*, 2003a). A single oral dose of bacteriophage CEV1 reduced intestinal levels of *E. coli* O157:H7 by two log units

within 2 days, when the animals were euthanized for examination of gut contents (Raya *et al.*, 2006). Multiple oral doses of mixtures or “cocktails” of different bacteriophages may be more effective for reducing *E. coli* O157:H7 shedding in animals (Bach *et al.*, 2009; Callaway *et al.*, 2008b).

Most studies have administered bacteriophages to animals orally by feed or water. However, some recent experiments have administered bacteriophages by direct application to the rectoanal junction as a means of targeting *E. coli* O157:H7 at its colonization site. As mentioned above, Sheng *et al.* (2006) both added bacteriophages to the drinking water and applied bacteriophages to the rectoanal junction mucosa; this treatment reduced but did not eliminate *E. coli* O157:H7 from the majority of the experimentally inoculated steers. Rozema *et al.* (2009) recently demonstrated that oral administration of multiple doses of a four-strain *E. coli* O157:H7-specific bacteriophage cocktail to cattle resulted in fewer *E. coli* O157:H7 positive samples and a lower mean shedding level of the pathogen, compared to rectal administration of the same bacteriophage treatment.

Additional work will be needed to develop and verify the use of *E. coli* O157:H7-infecting bacteriophages as a method to control *E. coli* O157:H7 in cattle. However, progress in the area is promising, and has identified a number of items for consideration. Continuous bacteriophage therapy has been suggested for the successful reduction of *E. coli* O157:H7 in cattle, provided that the targeted *E. coli* O157:H7 do not develop resistance (Rozema *et al.*, 2009). Niu *et al.* (2009a) demonstrated that lytic capability and host range are considerations when selecting bacteriophage, and further established that the use of bacteriophage cocktails is likely the most effective approach to address the resistance that some *E. coli* O157:H7 strains may have to some phages and/or the development of resistance by *E. coli* O157:H7 upon phage exposure.

D. Chlorate

Enterobacteriaceae is a large family of facultative anaerobic bacteria and includes many pathogens, such as *E. coli* O157:H7. These bacteria typically utilize oxygen for aerobic respiration, but when oxygen is absent such as in the gastrointestinal tract, they can perform fermentation. However, some of these bacteria, including *E. coli*, can continue to respire when alternative electron acceptors are available. Exploitation of nitrate reductase to convert chlorate to the lethal ion chlorite has been proposed to control *E. coli* O157:H7 in the gastrointestinal tract, with little harm to commensal anaerobic bacteria (Anderson *et al.*, 2000). Rapid reductions of 2–3 log₁₀ per gram for coliforms, generic *E. coli*, and *E. coli* O157:H7 were documented for rumen and fecal samples collected from *E. coli* O157:H7-inoculated cattle given water supplemented with chlorate (Callaway *et al.*,

2002). Chlorate treatments would likely be administered to cattle just prior to shipping (Anderson *et al.*, 2005), and the application of chlorate for this use is pending U.S. Food and Drug Administration review and approval.

E. Neomycin sulfate

Neomycin sulfate is an aminoglycoside antibiotic that is approved for use in cattle to treat enteric infections. While not approved for use to reduce *E. coli* O157:H7 in cattle, research indicates that neomycin sulfate could be an effective intervention. Administration at therapeutic doses reduced fecal shedding of *E. coli* O157:H7 compared to controls (Elder *et al.*, 2002; Keen *et al.*, 2006b; Woerner *et al.*, 2006b) and also reduced hide prevalence (Woerner *et al.*, 2006b). In contrast, supplementation of milk replacer with oxytetracycline and neomycin may increase the probability of *E. coli* O157:H7 shedding in very young calves (Alali *et al.*, 2004). Because reduction of the pathogen occurs rapidly, a proposed usage of neomycin sulfate is short-term administration to cattle just prior to harvest (Elder *et al.*, 2002; Keen *et al.*, 2006b). However, concerns about the development of antibiotic resistance may hinder the approval of neomycin sulfate for this use (Callaway *et al.*, 2009).

F. Other dietary supplements

Modifications of the cattle diet would be a preferred method to alter pathogen shedding, but to be successful, such modifications must provide long-term benefits without compromising animal productivity. Feeding hay to reduce pathogen shedding was proposed early (Diez-Gonzalez *et al.*, 1998) and has been much debated (Callaway *et al.*, 2003; Lejeune and Wetzel, 2007), but any benefits to reducing pathogen shedding are likely short-term. Feeding whole cottonseed has been associated with reduced *E. coli* O157:H7 shedding (Buchko *et al.*, 2000a; Garber *et al.*, 1995), but in other epidemiological studies cottonseed meal or whole cottonseed was not associated with changes in shedding of the pathogen (Dargatz *et al.*, 1997; Sargeant *et al.*, 2004).

A number of potential dietary supplements have been tested *in vitro* with variable success in reducing *E. coli* O157:H7. The rumen would be a target for most dietary interventions and *in vitro* tests with *E. coli* O157:H7-inoculated rumen fluid have shown antimicrobial capabilities for prebiotic sugars (de Vaux *et al.*, 2002), caprylic acid (Annamalai *et al.*, 2004), esculetin and esculin (Duncan *et al.*, 2004), and citrus peel and pulp (Callaway *et al.*, 2008a), whereas known rumen modifiers *Saccharomyces cerevisiae* (Bach *et al.*, 2003b) and dicarboxylic acids (Nisbet *et al.*, 2009) had no effect on pathogen reduction. Fecal incubations with esculetin and

esculin (Duncan *et al.*, 2004), *para*-coumaric, ferulic, and *trans*-cinnamic acids (Wells *et al.*, 2005), and sainfoin forage (Berard *et al.*, 2009) reduced levels of inoculated *E. coli* O157:H7. In animal studies, esculetin and esculin (Duncan *et al.*, 2004) reduced *E. coli* O157:H7 in inoculated animals, and sainfoin forage (Berard *et al.*, 2009) and tannin extracts (Min *et al.*, 2007) resulted in lower levels of generic *E. coli* in feces of treated animals. Orally administered egg yolk antibodies against *E. coli* O157:H7 were effective in reducing the pathogen shedding from inoculated lambs (Cook *et al.*, 2005). Efficacy studies with the above products in feedlot trials targeting *E. coli* O157:H7 have not been reported to date.

A product from brown seaweed, *Ascophyllum nodosum*, has been shown to reduce *E. coli* O157 shedding when fed at 2% dry matter intake in challenge studies (Bach *et al.*, 2008) and in feedlot trials (Braden *et al.*, 2004). The brown seaweed product also increased carcass marbling scores (Braden *et al.*, 2007) and although the antimicrobial extract of brown seaweed phlorotannin did reduce starch fermentation at high levels *in vivo* (Wang *et al.*, 2008), no effect on lamb growth was observed (Bach *et al.*, 2008).

G. Manure and cattle pen surface treatments

Most preharvest control research has focused on methods to reduce the prevalence and levels of shedding of *E. coli* O157:H7 by cattle. However, it is likely that environmental replication and persistence, along with amplification and shedding by cattle, combine to maintain *E. coli* O157:H7 in cattle production. Thus, strategies to reduce this organism may need to target both shedding by cattle and persistence in manure in order to break the transmission cycle of *E. coli* O157:H7. Information reporting direct fecal–oral transmission of this pathogen to cattle and work describing its long-term survivability in manures and feedlot surface soils further indicate a role for managing manure to reduce cattle exposures to *E. coli* O157:H7. Reducing *E. coli* O157:H7 from manures will have the additional benefit of reducing the potential for the environmental contamination that is associated with transmission of this pathogen from bovine manure to surface and groundwaters and food/feed crops. However, procedures to reduce pathogens from manures should also consider any potential impacts on the other health and nuisance problems that are linked to manure, namely volatile odor emissions and excess nutrients.

The periodic cleaning and disinfection of cattle pens has not been demonstrated to reduce *E. coli* O157:H7 in cattle, likely due to the constant replenishment of fresh feces on the pen floors (Elder and Keen, 1999; Folmer *et al.*, 2003). Providing dry bedding was associated with a reduced burden of *E. coli* O157 in cattle (Ellis-Iversen *et al.*, 2008); likewise, wet bedding was identified a risk factor for the pathogen (Ellis-Iversen *et al.*,

2007), which is consistent with other reports associating wet or muddy manures with higher *E. coli* O157:H7 incidence (Garber *et al.*, 1999; Smith *et al.*, 2001).

Treatment with carbonate and alkali has been demonstrated to inactivate *E. coli* in cattle manure (Arthurs *et al.*, 2001; Diez-Gonzalez *et al.*, 2000; Jarvis *et al.*, 2001; Park and Diez-Gonzalez, 2003). High pH is a critical feature, as the carbonate anion is responsible for the bactericidal activity (Diez-Gonzalez *et al.*, 2000). Park and Diez-Gonzalez (2003) further examined the abilities of alkali treatment of cattle manure with carbonate and ammonia to reduce *E. coli* O157:H7. Because of naturally present carbonate and ammonia in mixtures of feces and urine, pH adjustment with sodium hydroxide alone effectively reduced the pathogen. Similarly, the pathogen was inactivated when manure was supplemented with urea, because of an increase in pH as a result of the enhanced production of ammonia and carbonate from urea hydrolysis. Manure treatment with urea, sodium hydroxide, and/or sodium carbonate to reduce pathogens may be relatively simple and cost-effective, but additional studies are needed to determine the effectiveness of these treatments in the animal production environment.

Laboratory studies with plant essential oils including carvacrol, eugenol, and thymol, indicated that these antimicrobial compounds are not only effective for inhibiting odor emissions from livestock manure, but they can also reduce or eliminate *E. coli* and total coliforms (Varel and Miller, 2001, 2004). Thymol (from thyme oil) was incorporated into corn-cob granules to improve its stability and applied to feedlot pen surfaces (Varel *et al.*, 2006). Manure from thymol granule-treated pens had lower concentrations of both generic *E. coli* and coliforms in comparison to untreated control pens. Throughout an 8-week study, *E. coli* O157 was not recovered from manure in any of the thymol granule-treated pens, and was recovered repeatedly from only one untreated pen. In a separate study, direct application of thymol to feedlot pen surfaces reduced *E. coli* O157:H7 prevalence by 50% in all treated pens (Wells *et al.*, 2006).

Addition of urease inhibitors can prolong the retention of urea nitrogen in the manure, thereby improving its fertilizer value and reducing ammonia emissions (Varel *et al.*, 1999). However, by inhibiting urea hydrolysis to ammonia and carbon dioxide, urease inhibitors may enhance the survival of pathogens in manure (Park and Diez-Gonzalez, 2003). *In vitro* studies using slurries of cattle manure and urine indicated that addition of the urease inhibitor *N*-(*n*-butyl) thiophosphoric triamide (NBPT) can extend the survival of coliform bacteria in the manure (Varel *et al.*, 2007a). However, coliform bacteria were rapidly eliminated when the plant oil thymol was used in combination with NBPT in the manure slurries (Varel *et al.*, 2007a). Similarly, the application of NBPT with the plant oil extracts of linalool and pine oil on surfaces of feedlot pens

reduced generic *E. coli* and coliform bacteria in surface manure, in addition to conserving urea nitrogen and controlling odor production (Varel *et al.*, 2007b). Further work is needed to determine whether feedlot pen surface treatments with thymol or other essential oils that reduce *E. coli* O157:H7 in manure can also reduce the levels or prevalence of the pathogen in cattle.

In recent work, we examined the effects of pond ash-surfaced feedlot pens on the prevalence, levels, and persistence of *E. coli* O157:H7 in 128 beef cattle during an 84-day finishing period (Berry *et al.*, 2010). Pond ash is a low-cost byproduct of coal-fired electricity generation that provides a hard surface when packed into layers (ACAA, 2008). Benefits of pond ash as a feedlot pen surface include the provision of a solid base during wet weather, thus improving footing for cattle, an easier-to-clean surface, and it may also provide a cleaner area for cattle to rest in, thus alleviating some of the problems associated with muddy pens (dirty animals, loss of traction, stress, and effort expended for walking through mud). Given these potential advantages of pond ash in comparison to traditional soil surfaces, we further hypothesized that use of pond ash may affect the transmission of *E. coli* O157:H7 among cattle, or the ability of *E. coli* O157:H7 to persist in accumulated manure. The prevalence of *E. coli* O157:H7 in feces and on hides of the cattle on both pond ash- and soil-surfaced pens decreased during the study period, but there was no detectable effect of pen surface type. Similarly, no differences were seen in either the prevalence of *E. coli* O157:H7 or the levels of generic *E. coli* in feedlot surface manure in pens of cattle housed on soil- and pond ash-surfaced feedlot pens, nor in survival of *E. coli* O157:H7 and generic *E. coli* in surface manure from the two types of pen surfaces.

V. LINKING PREHARVEST AND POSTHARVEST REDUCTION OF *E. COLI* O157:H7

Numerous studies have demonstrated that processing practices and antimicrobial intervention procedures applied at slaughter, including hide washes, steam pasteurization, organic acid washes, hot water washes, or combinations of these treatments, substantially reduce *E. coli* O157:H7 from cattle carcasses (Barkocy-Gallagher *et al.*, 2003; Bosilevac *et al.*, 2005; Elder *et al.*, 2000; Woerner *et al.*, 2006a). Many of these same studies also have shown that the effectiveness of antimicrobial carcass interventions is improved by reducing the pathogen load at previous steps in the process (Arthur *et al.*, 2004; Brichta-Harhay *et al.*, 2008; Woerner *et al.*, 2006a). As mentioned above, high-level fecal shedding of *E. coli* O157:H7 is associated with increased hide contamination, and hides are an important source of beef carcass contamination at harvest (Arthur *et al.*, 2009;

Stephens *et al.*, 2009). Thus, it follows that pathogen reduction efforts applied throughout the animal production and processing chain should reduce the risk of *E. coli* O157:H7 occurrence in the final beef products.

However, recent studies suggest that any benefits of preharvest control efforts may be nullified by increases in *E. coli* O157:H7 infection and hide carriage of cattle that may occur during transportation and lairage. Arthur *et al.* (2007) found that both prevalence and levels of *E. coli* O157:H7 on cattle hides increased during transportation and lairage. Pulsed-field gel electrophoresis subtyping of isolates from cattle before and after transportation and from carcasses after processing revealed a large number of unique *E. coli* O157:H7 subtypes that were not detected at the feedlot, some of which were found in the transport trailers and many of which were likely a result of contamination from the lairage environment (Arthur *et al.*, 2007). Subsequent work observed similar increases in *E. coli* O157:H7 hide prevalence from the feedlot through transport and lairage, and the pathogen was recovered from 64% of transport trailers and 60% of samples collected from the lairage environment (Arthur *et al.*, 2008). Molecular subtyping of *E. coli* O157:H7 isolates indicated that cattle hide contamination that occurred in lairage accounted for a larger proportion of the hide and carcass contamination than did contamination from the feedlot (Arthur *et al.*, 2008). Similarly, Mather *et al.* (2008) found that 84% of cattle at slaughter had *E. coli* O157 subtypes on their hides that did not match subtypes found previously on the farm of origin.

In contrast, Fegan *et al.* (2009) did not observe increases in either prevalence or levels of *E. coli* O157 in feces or on hides as a result of transportation and lairage. *E. coli* O157 prevalence in feces were similar at the feedlot (18%) and after slaughter (12%), and hide prevalence decreased from 31% at the feedlot to 4% after transportation and lairage. Subtyping isolates by pulsed-field gel electrophoresis showed that all *E. coli* O157 from hides and feces at slaughter were of the same subtype as those collected at the feedlot. Minihan *et al.* (2003) did not examine hides, but also did not see an increase in *E. coli* O157 fecal shedding by cattle as a result of transportation and lairage. Reicks *et al.* (2007) found *E. coli* O157:H7 on less than 2% of feedlot cattle hides both before and after shipping.

Risk factors for *E. coli* O157:H7 hide contamination during transportation and lairage included holding cattle in *E. coli* O157:H7 positive lairage pens, holding cattle in feces-contaminated pens, and transportation for distances greater than 160.9 km (Dewell *et al.*, 2008). Mather *et al.* (2008) identified transport to the processing plant by a commercial hauler, as opposed to the farmer, as a risk factor for cross-contamination of cattle hides. Odds of previsceration carcasses being positive for *E. coli* O157:H7 were higher within truckloads of cattle containing at least one animal with fecal *E. coli* O157:H7, and were particularly high when at least one high-level shedding animal was within the truckload (Fox *et al.*, 2008b).

While not in full agreement, results of the cited studies indicate that *E. coli* O157:H7 prevalence or numbers in and on cattle during transportation or in lairage can increase as a result of contact with one another, or with contaminated feces, transport trailers, or holding pens at lairage. These observations suggest that preservation of *E. coli* O157:H7 reduction benefits achieved on the feedlot or farm by preharvest control strategies would require the wide adoption and practice of these procedures, and that interventions are needed to limit cattle contamination with this pathogen during transportation and lairage.

VI. CONCLUSIONS AND FUTURE PROSPECTS

In the nearly 30 years since *E. coli* O157:H7 became recognized as a foodborne pathogen, research has revealed much about the occurrence of this pathogen in cattle, the production environment, and the factors that affect its prevalence, levels, and persistence in cattle. Despite this, questions remain unanswered and foodborne disease caused by this pathogen continues to occur. Successful preharvest interventions that have been examined to date, both those in current use and those in experimental development, can reduce but do not eliminate *E. coli* O157:H7 in cattle. Indeed, it seems clear that elimination of *E. coli* O157:H7 from cattle is unrealistic; however, evidence indicates that minimizing the levels or concentrations of the pathogen in cattle production will have substantial impact on its prevalence in cattle, the success of postharvest intervention efforts to reduce it, and on its occurrence in final beef products. Furthermore, in addition to lowering the risk of disease associated with beef consumption, reducing the numbers and persistence of *E. coli* O157:H7 in cattle should also reduce environmental contamination, thereby lowering the risk of water and produce contamination. Thus, research on the preharvest control of this pathogen must continue.

Determination of factors that cause super-shedding of *E. coli* O157:H7 by some cattle are expected to reveal strategies to mitigate this occurrence, and in combination with procedures that reduce the persistence of *E. coli* O157:H7 in the production environment, should break the infection cycle. Hypotheses regarding the effect of super-shedders on *E. coli* O157:H7 prevalence in cattle may have economic implications for the cattle industry, and need to be confirmed. The concept that removing or eliminating high-level shedders will reduce *E. coli* O157:H7 in cattle suggests that limited resources or expensive control measures (e.g., vaccines) can be targeted at these animals, with the benefit extending to the entire herd.

Information regarding the significance of *E. coli* O157:H7 super-shedders and pathogen persistence to cattle infection and environmental contamination suggest that the food safety goals should be reductions in

the levels or loads of the pathogen. Thus, studies measuring the effects of *E. coli* O157:H7 control procedures should collect not only prevalence data but also quantitative data. The use of enumerative methods has provided more precise evaluation of relative impact of various factors affecting shedding, prevalence, and persistence of *E. coli* O157:H7, and the combination of prevalence and concentration data provides information regarding both the distribution and the magnitude of the pathogen. Furthermore, microbial risk assessments require data not only on the occurrence, but also the concentration of *E. coli* O157:H7 in cattle and the environment, for determination of transmission risks from these sources.

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