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Escherichia coli O157:H7 in beef cattle: on farm contamination and pre-slaughter control methods

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Abstract

This paper addresses food safety in beef cattle production, with particular emphasis on factors that affect the prevalence of *Escherichia coli* O157:H7 in beef cattle and on control methods that have been investigated. Product recalls and foodborne diseases due to this organism continue to occur even though control measures have been under investigation for over 20 years. Most meatborne outbreaks are due to improper food handling practices and consumption of undercooked meat. However, the majority of pathogenic bacteria that can spread at slaughter by cross-contamination can be traced back to the farm rather than originating from the slaughter plant. This would ideally require the adoption of rigorous on-farm intervention strategies to mitigate risks at the farm level. On-farm strategies to control and reduce *E. coli* O157:H7 at the farm level will reduce the risk of carcass contamination at slaughter and processing facilities although they will not eliminate *E. coli* O157:H7. The most successful strategy for reducing the risk of contamination of beef and beef products will involve the implementation of both pre- and post-harvest measures.

Keywords: beef cattle, *Escherichia coli* O157:H7, food safety, on-farm intervention strategies, management, vaccine, probiotics, bacteriophage

Introduction

Microorganisms are widely distributed in animals and in foods of animal origin. The major causes of concern and product recalls associated with meat and poultry products are *Escherichia coli* O157:H7, *Salmonella enteritidis*, *Campylobacter jejuni* and *Listeria monocytogenes* (Sofos, 2008). These pathogens are found in animal feces (Hutchinson *et al.*, 2005) and contamination of carcasses and food products by animal feces is the major method for transmitting foodborne pathogens to consumers (Oliver *et al.*, 2008). Although foodborne outbreaks in recent years appear to have shifted from being primarily associated with foods of animal origin to increasingly being associated with fresh produce (CDC, 2008), shellfish (Pontrelli *et al.*, 2008) and ingredients (e.g. peanut butter) (CDC, 2009), product recalls and food-borne outbreaks indicate that meat safety continues to be a concern and challenge for food and health authorities and industries. Intervention is possible at many points along the production chain, but on-farm control points are likely to be most cost-effective (Humphrey *et al.*, 2007). Recently, the U.S. Department of Agriculture (USDA) awarded a $2.5 million grant to a university to develop strategies to reduce the shedding or release of Shiga toxin-producing *E. coli* by cattle (STEC) (USDA, 2011). This important investment indicates the priority being assigned to reducing the microbial load at the farm level in order to prevent contamination further down the food chain.

Even though most bacterial foodborne outbreaks were traced to improper food handling practices, Nørrung

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and Buncic (2008) noted that the original sources of foodborne pathogens that cause most meatborne bacterial diseases are asymptomatic farm animals that carry and shed pathogens in the feces. In many cases, farmed ruminants carrying zoonotic pathogens in the gastrointestinal tract show no signs of infection (Adam and Brülisauer, 2010). Transfer of bacteria from the hide and gut contents to the carcass can occur during hide removal and evisceration in the abattoir (McEvoy *et al.*, 2000; Huffman, 2002; Reid *et al.*, 2002). The majority of pathogenic bacteria that can spread at slaughter by cross-contamination were traced back to production on the farm rather than originating from the slaughter plant (Autio *et al.*, 2000; Wegener *et al.*, 2003). The contamination cycle in food-producing animals occurs through the ingestion of feeds and water that are contaminated with feces. The use of untreated manure as fertilizer and spread of slurry on grazing fields also contribute to the spread of microbial pathogens. Stresses on animals due to poor management (Nørrung and Buncic, 2008), and quantity and quality of animal feed increase the susceptibility to infections and the shedding of foodborne pathogens (Adam and Brülisauer, 2010). Oliver *et al.* (2008) suggested that all these environmental and management factors must be considered when identifying farm practices and critical control points on the farm where contamination occurs.

Elder *et al.* (2000) reported that fecal shedding by cattle is correlated with carcass contamination. This association between fecal prevalence and carcass contamination indicates a role for control of microbial pathogens in cattle on the farm to reduce the risk of human infection from ingestion of undercooked beef or cross-contamination of other foods. Traditionally, much of the research effort was aimed at improving the safety of meat products after slaughter and during processing (Elder *et al.*, 2000) and several post-slaughter steps that reduce the level and frequency of *E. coli* O157:H7 on beef have been implemented. However, consumers are still sickened by foodborne disease outbreaks. Hence, increased emphasis is being laid on pre-slaughter intervention strategies (Callaway *et al.*, 2003). Hynes and Wachsmuth (2000) assert that 'strategies that reduce foodborne pathogenic bacterial populations in the animal prior to slaughter could produce the most significant reduction in human exposures to the organism and therefore reduction in related illnesses and deaths. It should be noted that activities at the farm level that affect excretion of *E. coli* O157:H7 by cattle will affect not only fecal contamination of beef but will also affect contamination of the environment, including water that receives runoff from farms. This environmental contamination frequently leads to human infections through bathing, and irrigation and washing of fruits and vegetables.

LeJeune and Wetzel (2007) also reported that intervention strategies that target the pathogen in live animals on the farm before slaughter may have the largest impact on improving beef safety. However, Sofos (2008) noted that pathogen control in animals during the pre-harvest stage is difficult due to limitations in the existing scientific information. It is recognized that on-farm interventions are not likely to eliminate *E. coli* O157:H7 from cattle presented for slaughter (Arthur *et al.*, 2009). An understanding of the possible sources of on-farm infection is important for effective control. For example, Davies (2005) stated that once pathogens are introduced onto the farm, it is important to understand their spread, the involvement of other farm animals and wildlife, contamination of equipment and personnel, and airborne spread and survival in environmental niches. Only then can the organisms be effectively controlled. Hence, this paper seeks to review the scientific literature reporting potential sources of on-farm infections among food-producing animals and intervention strategies adopted to reduce the risk of contamination.

Table 1. Relative frequency of beef as the implicated source of *E. coli* outbreaks reported internationally between 1988 and 2007 (Greig and Ravel, 2009)

Food source	Proportion (%)
Produce	19.5
Multi-ingredient foods	11.8
Seafood	0.5
Beef	44.2
Pork	0.5
Dairy products	9.8
Chicken	1.0
Other meats	6.9
Bakery items	1.0
Beverages	4.4
Turkey and other poultry	0.3

Foodborne outbreaks traced to beef products

Table 1 shows the relative frequency of foodborne outbreaks in beef due to *E. coli* O157:H7. Foodborne disease caused by *E. coli* O157:H7 infection decreased by 44% in the US in 2010 compared to 1996–1998. This is translated as $\leq$1 case of *E. coli* per 100,000 and reaching this goal was a huge success (Table 2). The goal for 2020 is a 50% reduction compared to the 3-year baseline period of 2006–2008. Many factors likely contributed to the decrease in incidence of *E. coli* O157:H7 infections, such as improved detection and investigation of outbreaks which leads to prompt recalls and enhanced knowledge on sources of contamination (CDC, 2011). Interventions applied pre- and post-slaughter also contributed to the decline in *E. coli* O157 infections (Gyles, 2007; CDC, 2011).

Cattle are the major reservoir for *E. coli* O157:H7 (Nørrung and Buncic, 2008). It is estimated that 20 STEC O157 illnesses occur for every one that is reported

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Table 2. Number of STEC O157:H7 infections per 100,000 persons per year in the US (CDC, 2011)

Year	1996–1998	2006–2–08	2010	2020 Objective
Number of infections per 100,000 persons	2/100,000	1.2/100,000	≤1/100,000	0.6/100,000

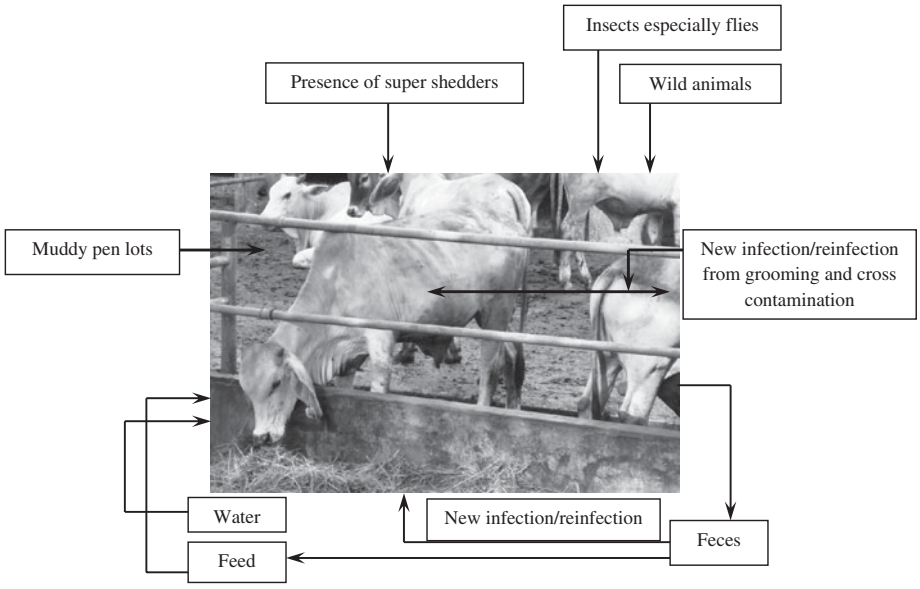

Fig. 1. Potential on-farm contamination of beef cattle with *E. coli* O157:H7.

(Mead *et al.*, 1999). *E. coli* O157:H7 has been more researched than other serotypes of STEC because it is the serotype most frequently involved in outbreaks and in severe disease. Its ability to ferment sorbitol is a convenient marker for screening for this pathogen and has facilitated detection of this pathogen in patients and in animal feces (Bettelheim, 2007).

Sources and transmission routes of *E. coli* O157:H7 in beef cattle

There are several recent reviews that critically discuss the risk factors and the transmission and prevalence of *E. coli* O157:H7 in cattle (Oliver *et al.*, 2008; Ellis-Iversen *et al.*, 2009; Berry and Wells, 2010). The prevalence of *E. coli* in water, feed, hide and soil are of major importance. We have illustrated and summarized some of the sources and transmission routes of *E. coli* O157:H7 in beef cattle (Fig. 1).

Water and feed

LeJeune *et al.* (2001) demonstrated that cattle water troughs can be reservoirs of *E. coli* O157:H7 on farms and serve as a source of infection for cattle. This is in agreement with Hancock *et al.* (1998) who had earlier reported that *E. coli* O157:H7 were able to survive in water trough sediments for at least 4 months and appeared to multiply especially in warm weather. However, improved water trough hygiene did not reduce the risk of *E. coli* O157:H7 in young cattle (Ellis-Iversen *et al.*, 2008, 2009). Similar to water contamination, the hygiene of animal feed plays a key role in microbial contamination in livestock (Crump *et al.*, 2002) since feed can be a vehicle for transmitting *E. coli* O157:H7 to cattle (Hancock *et al.*, 2001; Davis *et al.*, 2003; Horchner *et al.*, 2006). Fenlon and Wilson (2000) also demonstrated that *E. coli* can multiply in feed. *E. coli* O157:H7 inoculated in laboratory silage (made from rye grass) increased from an initial level of 10^3 to 10^7 colony-forming units (CFU) g^{-1} within 13 days. *E. coli* O157:H7 was isolated from the oral cavities of 74.8% of cattle examined and it has been suggested that feed may be contaminated by *E. coli* O157:H7 from cattle saliva (Keene and Elder, 2002). Cattle return rumen contents to their mouths to be chewed again and further digested and this may be the most probable source of *E. coli* O157:H7 in the animals' mouths (Tkalcic *et al.*, 2003). Other possible sources include fecal contamination by wildlife (Fischer *et al.*, 2001; Renter *et al.*, 2001), including birds (Shere *et al.*, 1998; Nielsen *et al.*, 2004), rodents (Nielsen *et al.*, 2004) and insects (Ahmad *et al.*, 2007). However, LeJeune *et al.* (2006) did

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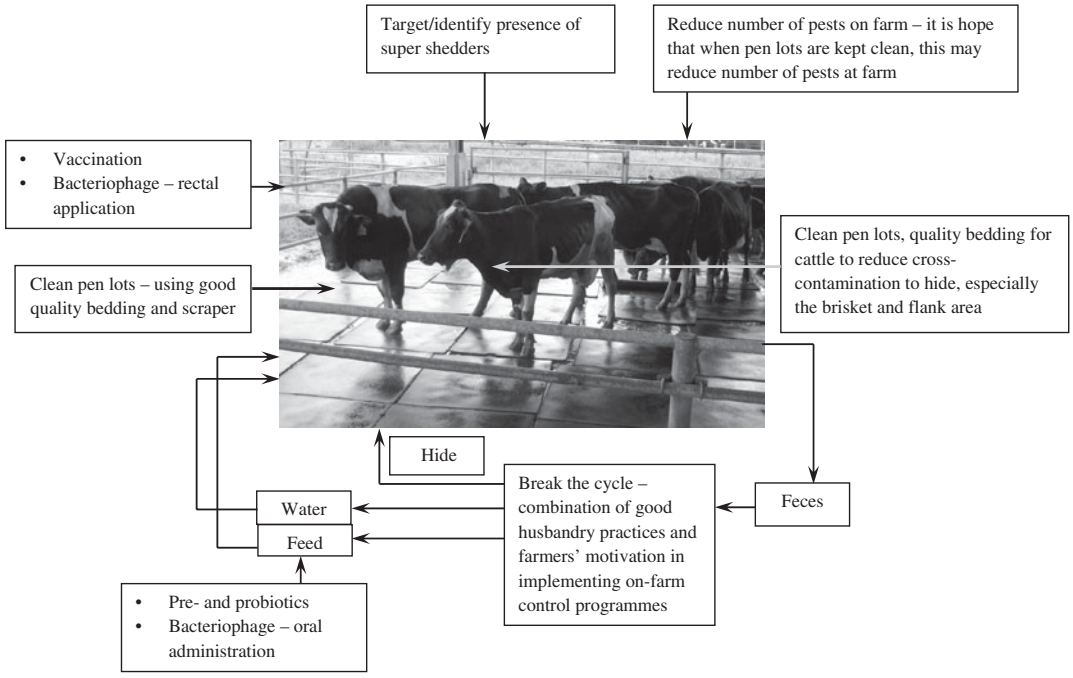

Fig. 2. On-farm control measures that have been suggested.

not find significant correlation between the magnitude of feed contamination and *E. coli* O157:H7 prevalence in cattle.

Diez-Gonzalez *et al.* (1998) argued that grain-diet promoted acid production in the colon, which leads to increased acid-resistant generic *E. coli* strains in the faeces. The authors demonstrated that hay-fed cattle had a lower concentration of volatile fatty acids in their colons and that acid shock killed more than 99.99% of the *E. coli*. When diets were supplemented with grain, acids accumulated, colonic pH declined and this selectively favored *E. coli* resistant to extreme acid shock. There has been much debate since the intervention of switching to a forage-based diet before slaughter was first described. It was noted that the authors did not investigate *E. coli* O157:H7 and that the results with generic *E. coli* could not be extrapolated to *E. coli* O157:H7. Although grain-fed cattle harbored more acid-resistant generic *E. coli* than did forage-fed cattle (Diez-Gonzalez *et al.*, 1998; Hovde *et al.*, 1999), the acid resistant *E. coli* O157:H7 was not suppressed by a forage diet (Hovde *et al.*, 1999; Grauke *et al.*, 2002). Van Baale *et al.* (2004) also demonstrated that feeding forage actually increased the shedding of *E. coli* O157:H7 in cattle. Cattle fed forage diets were culture positive for *E. coli* O157:H7 in the feces for longer duration than cattle fed a grain diet. Judging from the various dietary interventions, Callaway *et al.* (2009) emphasized that dietary manipulations is a potentially powerful method to reduce *E. coli* populations in cattle but further research is essential to clarify the effect of different diets on the bovine gastrointestinal system (Wood *et al.*, 2006).

Besides implementing HACCP in animal feed-processing plants, management of feed in the farm must involve reducing exposure to wildlife excreta (Daniels *et al.*, 2003). Oliver *et al.* (2008) concluded that the contamination cycle occurs when cattle ingest contaminated feed and water, followed by shedding of foodborne pathogens in feces, which then contaminate feeds and animal drinking water, causing new infections and reinfection of animals. In order to break this infection–reinfection cycle (Fig. 2), on-farm foodborne control programms based on the critical points of transmission can be designed to reduce the introduction of foodborne pathogens into processing plants and the human food chain (Sargeant *et al.*, 2000; Oliver *et al.*, 2008). Methods for both pre-harvest and post-harvest control of *E. coli* O157:H7 have been widely studied, but the development of a simple and universal effective mitigation strategy remains elusive. The most successful strategy will involve the implementation of both pre- and post-harvest measures (Wood *et al.*, 2007).

Role of hide for pathogen transmission

Hide cleanliness and prevalence of foodborne pathogens may be associated with the pen feedlot condition. Smith *et al.* (2001) observed that higher percentages of cattle in muddy feedlot pens shed *E. coli* O157:H7 compared to cattle in normal pen conditions. These researchers reasoned that the muddy feedlot pens may facilitate fecal – oral transmission. Similarly, Cobbold and Desmarchelier (2002) suggested that pen floors and hides

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were the main source of STEC transmission to dairy calves. Bach *et al.* (2005) also suggested that faeces on pen floors are a more significant source of infection than are feed or drinking water. Reid *et al.* (2002) found that the brisket area contains the highest concentration of bacteria on the hide compared to the rump or flank area. This is in agreement with McEvoy *et al.* (2000) who demonstrated that the total viable bacterial counts were significantly higher at the brisket. This may be attributable to the fact that the brisket area is in contact with the floor when cattle are resting. This is also the site where the initial cut is made during the hide-removal process and there is a high probability of transferring pathogens from the hide to the carcass (McEvoy *et al.*, 2000). High-level fecal shedding of *E. coli* O157:H7 has also been linked to increased hide contamination (Arthur *et al.*, 2009; Stephens *et al.*, 2009).

There is evidence that super shedding cattle have a large impact on the overall contamination of animals due to the increased animal density and confined spaces associated with farm and lairage environments (Arthur *et al.*, 2010). Cattle that excrete exceptionally high numbers of *E. coli* O157 have been referred to as high-level shedders or 'super-shedders' (Chase-Topping *et al.*, 2007; Berry and Wells, 2010). High shedding has been defined as counts of *E. coli* O157:H7 that are $\geq 10^3$ (Low *et al.*, 2005) or 10^4 CFU g^{-1} of feces (Omisakin *et al.*, 2003; Ogden *et al.*, 2004). Matthews *et al.* (2006) reported that 20% of the *E. coli* O157:H7 infected cattle were responsible for 80% of the transmission of the organism in Scottish cattle farms. Another study reported similar findings, where 9% of the animals shedding *E. coli* O157:H7 produced over 96% of the total *E. coli* O157:H7 fecal load for the group (Omisakin *et al.*, 2003). Cobbold *et al.* (2007) showed that the cattle that did not shed *E. coli* O157:H7 were five times more likely to be housed in a pen that did not contain a super-shedder. Matthews *et al.* (2006) suggested that the spread of *E. coli* O157:H7 between cattle could be controlled if one could prevent super shedding in the 5% of individuals that are the main source of contamination. Ultimately, significant reductions may be made by targeting the super-shedders (Chase-Topping *et al.*, 2008). Measures that reduce the carriage and shedding of *E. coli* O157:H7 also have the potential to reduce secondary transmission through feed, drinking water or direct contact and grooming (Gyles, 2007). Chase-Topping *et al.* (2008) presented a comprehensive discussion of super-shedding and the risk for human infection.

Wild and domestic animals and insects

Wildlife fecal contamination can serve as a potential source of infection to livestock (Daniels *et al.*, 2003). Animals that have been shown to carry *E. coli* O157:H7 include, but are not limited to, wild deer (Sargeant *et al.*, 1999; Fischer *et al.*, 2001; Renter *et al.*, 2001), rats (Cizek *et al.*, 1999; Nielsen *et al.*, 2004) and raccoons (Shere *et al.*, 1998). Birds are another singular and important source of transmission and birds found positive for *E. coli* O157:H7 include pigeons (Shere *et al.*, 1998), gulls (Wallace *et al.*, 1997) and starlings (Nielsen *et al.*, 2004). A study by Scaife *et al.* (2006) in Norfolk, UK found 20.62% (20/97) of fecal samples collected from wild rabbits were positive for STEC O157. Ahmad *et al.* (2007) showed that houseflies are capable of transmitting *E. coli* O157:H7 to cattle. Fecal samples from all calves exposed to inoculated flies were positive. The pathogen counts were as high as 1.5×10^5 CFU per fly. This high concentration of *E. coli* O157:H7 suggested that houseflies are not simply mechanical vectors, but that the pathogen likely multiplied in the gastrointestinal tract of the houseflies (Alam and Zurek, 2004). Although cattle are considered the main reservoir of *E. coli* O157:H7, strains of *E. coli* O157:H7 may be introduced into cattle populations through feed (Daniels *et al.*, 2003) and water contaminated with the feces of wild and domestic animals (Wetzel and LeJeune, 2006). In most instances it is impossible to keep wild animals out of the farm but it is important for farms and farm workers to be aware that wild animals can also act as vectors for infection via the fecal–oral route.

On-farm intervention strategies

The distribution of *E. coli* O157:H7, its persistence in the environment, and its ability to infect and reinfect cattle and wildlife make eradication an unrealistic goal. Traditional means of controlling infectious agents, such as eradication, involving testing and removal of carrier animals are not feasible for this pathogen. An achievable objective is to reduce the magnitude or prevalence of *E. coli* O157:H7 in feces and to break the contamination cycle (LeJeune and Wetzel, 2007). Koohmaraie *et al.* (2005, 2007) suggested that the post-harvest is the most logical and effective step to maximally reduce *E. coli* O157:H7 (and other pathogens) but it is evident that reductions in the pre-harvest stages will reduce environmental contamination and enhance the effectiveness of post-harvest measures.

Farm management practices – especially the maintenance of feed and water may be the most practical means of reducing infectious agents in cattle (Hancock *et al.*, 2001). Oliver *et al.* (2008) suggested that all environmental and management factors must be considered when identifying farm practices and critical control points on the farm where contamination occurs. According to Collins and Wall (2004), it is the primary producer who should take all reasonable measures to reduce the entry and prevalence of *E. coli* O157 on his/her farm. They need to adopt approaches on the farm with the objective of eliminating or minimizing carriage and shedding of

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zoonotic agents by cattle. However, although some measures have shown promise it is difficult to pinpoint a single practice to a producer or a feedlot operator that can be predicted to consistently reduce the prevalence and/or concentration of *E. coli* O157:H7 in cattle in a cost-effective manner (Koohmaraie *et al.*, 2005). Regardless of the challenges, Loneragan and Brashears (2005) reported that the potential of on-farm control exists. Table 3 shows a summary of some of the control measures tested *in vitro* or in animal trials and on farms. Some have been effective under artificial conditions but require further investigation to evaluate the method and its feasibility in implementation at the farm level. We have also illustrated and summarized some of the management measures that have been tested in animal trials and at the farm level to reduce and control *E. coli* O157:H7 in beef cattle (Fig. 2).

Feed

A number of studies have identified animal feed as a potential source of infection of cattle with STEC O157 (Hancock *et al.*, 2001; Van Donkersgoed *et al.*, 2001; Dodd *et al.*, 2003). In 2004, Codex Alimentarius introduced the Code of Practice on Good Animal Feeding to establish a feed safety system for food-producing animals. The objective of the Code is to help ensure the safety of food through adherence to good animal feeding practices at the farm and good manufacturing practices during the processing and handling of feed and feed ingredients. It also states that 'where appropriate, Hazard Analysis Critical Control Point (HACCP) principles should be followed to control hazards that may occur'.

Dietary intervention has been suggested to offer a simple and practical means of reducing the prevalence of *E. coli* O157:H7 in the hindgut (Fox *et al.*, 2007). Berg *et al.* (2004) showed that cattle fed corn-based diets shed more generic *E. coli* than do cattle fed barley-based finishing diets. The more extensively cereal grains were processed, the more starch was digested in the rumen and this reduced the amount entering the lower digestive tract (Huntington, 1997). Since corn is less digestible in the rumen compared to barley (Huntington, 1997), this provided more undigested starch in the large intestine and resulted in increased fermentation and reduced fecal pH. Corn-fed cattle were found to have a lower mean fecal pH value (pH 5.85) compared to barley-fed cattle (pH 6.51) (Buchko *et al.*, 2000; Berg *et al.*, 2004). Feeding dry-rolled grain diet to cattle reduced the prevalence of *E. coli* O157:H7 by 35% as compared to steam-flaked grain diet. It is possible that dry-rolling allows more substrate to reach the hindgut where it increased fermentation and volatile fatty acid production and made the hindgut inhospitable to the survival of *E. coli* O157:H7 (Fox *et al.*, 2007; Depenbusch *et al.*, 2008). However, a number of studies demonstrated that this approach is not likely to be effective for *E. coli* O157:H7.

In calves, increased risk has been associated with feeding colostrum from a bucket compared to suckling. The reduced risk among calves that did suckle colostrum from the mother could be explained by the increased protection from maternal antibodies (Rugbjerg *et al.*, 2003). The researchers hypothesized that calves that suckle colostrum from the cow and stayed longer with the mother were in some way protected from infection with *E. coli* O157. However, this needs to be confirmed by further studies.

The fermentation of cereal grains to produce ethanol results in a co-product called distillers' grains (DG). The co-product is fed either as wet distillers' grain (WDG) (approximately 30% dry matter) or dried distillers' grains (DDG) (approximately 90% dry matter) (Spiehs *et al.*, 2002). DG were shown to increase daily weight gain in finishing cattle due to the condensed nutrients and hence were used in ruminant diets (Ham *et al.*, 1994). Cattle fed diets including 25% of DDG or 40% of WDG with solubles (WDGS) had a higher prevalence of *E. coli* O157:H7 in their feces. It is possible that (i) feeding dried distillers grains results in decreased starch concentration in the hindgut, which may alter the ecology and favor the growth of *E. coli* O157:H7 or (ii) components of the brewers' grain may stimulate the bacterial growth (Jacob *et al.*, 2008; Wells *et al.*, 2009). Cattle fed 20 or 40% of WDGS were also found to have prolonged survival of inoculated *E. coli* O157:H7 compared to those fed 0% WDGS. The slurries obtained from cattle fed 20 or 40% WDGS had lower concentrations of L-lactate and pH values between 6.0 and 8.0 (Varel *et al.*, 2008). L-lactate has a significant antimicrobial effect on *E. coli* O157:H7 as well as non-O157 *E. coli* (McWilliam Leitch and Stewart, 2002).

Essential oils have been shown to inhibit foodborne pathogens in pure culture (Burt, 2004). The addition of plant phenolic acids such as cinnamic acid, coumaric acid and ferulic acid to feces increased the death rate of *E. coli* O157:H7 (Wells *et al.*, 2005). Addition of orange peel and pulp to inoculated ruminal fluid reduced *E. coli* O157:H7 from 10^5 to 10^2 CFU ml^{-1}. This may be the result of the antimicrobial action of essential oils such as limonene found in the peel (Callaway *et al.*, 2008a). It is still unknown as to which constituents or mixtures of essential oils are responsible for their antimicrobial activity (Espina *et al.*, 2011). The major chemical component of most citrus oils is limonene with sweet orange containing 68–98% and lemon 45–76% (Svoboda and Greenaway, 2003). Further research is still needed to determine the mechanisms of action and whether the antimicrobial activity can be expressed in the livestock's lower gastrointestinal tract (Callaway *et al.*, 2008a). In addition, candidate plant compounds with antimicrobial activity or grasses used as cattle forages, which contain phenolic acids can be used as potential dietary additives or manure treatments (Wells *et al.*, 2005). Doyle and Erickson (2011) suggested that the active components of antimicrobial

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Table 3. On-farm *E. coli* O157:H7 control methods investigated in experimental animal trials or on farms

Type of on-farm control method	Observed effects	Currently used on farm	References
Diet manipulation			
Forage containing sainfoin	Marginal reduction in shedding	No	Berard *et al.* (2009)
Dry-rolled or steam-flaked grain-based diet	Feeding dry-rolled grains reduce *E. coli* O157:H7 prevalence by 35%	No	Fox *et al.* (2007)
Addition of citrus products	Reduced *E. coli* O157:H7 from 10^5 to 10^2 CFU ml^{-1}; requires further investigation	No	Callaway *et al.* (2008a)
Probiotics and direct-fed microbials			
L. acidophilus NP-51	Reduced shedding of *E. coli* O157:H7	Yes	Brashears *et al.* (2003a), Younts-Dahl *et al.* (2004), Loneragan and Brashears (2005), Peterson *et al.* (2007a)
L. acidophilus NP-51 and *P. freudenreichii*	Probability of recovery of *E. coli* O157:H7 from the feces of treated and control was statistically different at 34 and 66%	Yes	Tabe *et al.* (2008)
Colicin E7-producing *E. coli*	Overall reduction of 1.1 $\log_{10}$ CFU g^{-1} of *E. coli* O157:H7	No	Schamberger *et al.* (2004)
Prebiotics	Limited due to the ability of ruminants to digest most prebiotics	No	Callaway *et al.* (2008b), Doyle and Erickson (2011)
Bacteriophage therapy			
	A higher prevalence of phage in fecal/water samples was associated with reduced prevalence of *E. coli* O157:H7	No	Niu *et al.* (2009a)
Phage application to rectoanal junction of steers and supplying phage in drinking water	Reduced average number of *E. coli* O157:H7 among phage treated group compared to control ($p<0.05$)	No	Sheng *et al.* (2006)
Oral and rectal administration of phage	Lower shedding of *E. coli* O157:H7 in orally treated group.	No	Rozema *et al.* (2009)
Oral administration of encapsulated bacteriophage	Did not reduce shedding of *E. coli* O157:H7	No	Stanford *et al.* (2010)
Administration of vaccines/immunization			
Vaccination with type-III secreted proteins	Reduced prevalence, duration and magnitude of *E. coli* O157:H7 fecal shedding	Yes	Potter *et al.* (2004)
Two dose vaccination regime	Reduce colonization of *E. coli* O157:H7 in rectal	Yes	Peterson *et al.* (2007b)
Vaccine targeting SRP proteins	Vaccination with 3 doses result in 2 log reduction of *E. coli* O157 in feces	Yes (conditional license for use in US)	Thomson *et al.* (2009)
Husbandry			
Providing clean and dry bedding	Yes	Yes	Ellis-Iversen *et al.* (2007, 2008)
Chlorination of water	Marginal, poor palatability	No	LeJeune *et al.* (2004), Zhao *et al.* (2006)
Addition of phenolic acids to feces	Increased death rate (8–12 fold) of *E. coli* O157:H7	No	Wells *et al.* (2005)

compounds may not be reaching the *E. coli* colonization sites in animals; hence, encapsulation of these ingredients may warrant further investigation. Numerous studies have been carried out on dietary interventions to determine the optimum method of reducing the prevalence of *E. coli* O157:H7 in beef cattle. Since *E. coli* O157:H7 is a normal flora of cattle it is a daunting task to reduce its presence in the intestine.

Probiotics and direct-fed microbials

Probiotics or direct-fed microbials are preparations of live bacteria fed to a host to elicit beneficial health effects in the host (Schrezenmeir and de Vrese, 2001). Several lactic acid bacteria (LAB), most commonly *Lactobacillus*, *Enterococcus* and *Streptococcus*, have been tested as probiotic agents or competitive exclusion products (CEP) for livestock (Brashears *et al.*, 2003b). Competitive exclusion cultures consist of a mixture of undefined microbes and are usually isolated from the gastrointestinal tract of the animal species that will be treated, while probiotics are well-defined strains that have been cultured separately prior to application (Doyle and Erickson, 2011).

The genus *Lactobacillus* is one of the most commonly used genera of probiotic organisms added to a range of feeds (Gaggìa *et al.*, 2010). A specific strain, *Lactobacillus acidophilus* NP51, reduced the prevalence of *E. coli* O157:H7 by 49% in animals receiving NP51 compared to controls (Brashears *et al.*, 2003a). Peterson *et al.* (2007a) reported that by administering *L. acidophilus* strain NP51 in feed daily for 2 years, fecal shedding of *E. coli* O157:H7 decreased by 35% in beef cattle. In another study, steers fed a standard steam-flaked corn-based finishing diet containing *L. acidophilus* NP51 showed a reduction of *E. coli* O157:H7 fecal shedding by 57% (Younts-Dahl *et al.*, 2004) while a combination of *L. acidophilus* NP51 and *Propionibacterium freudenreichii* reduced fecal shedding of *E. coli* O157 by 32% compared to the control group (Tabe *et al.*, 2008). Selected cultures containing *E. coli* strains whose colicins killed *E. coli* O157:H7 (Schamberger and Diez-Gonzalez, 2002) or mixtures of probiotic *E. coli* (Tkalcic *et al.*, 2003; Zhao *et al.*, 2003) have also been tested for probiotic potential against *E. coli* O157:H7. However, bacteria can become resistant to the antimicrobial mechanisms of probiotic organisms. Laboratory studies indicated that *E. coli* O157:H7 can become resistant to individual colicins; hence, multiple-colicinogenic strains may be required for effective treatments (Schamberger and Diez-Gonzalez, 2005).

The inhibition of *E. coli* O157:H7 by probiotics may result from the decrease in pH due to the production of organic acids by LAB. It is also speculated that other factors such as production of bacteriocins, hydrogen peroxide, low-molecular-weight metabolites such as diacetyl and CO_2, or enzymes by LAB contribute to the inhibition (Brashears *et al.*, 2003b). Fujiwara *et al.* (1997) revealed that bifidobacteria produce a proteinaceous molecule(s) which prevents the binding of *E. coli* Pb176, an enterotoxigenic *E. coli* (ETEC) strain to intestinal mucosa. Studies by Medellin-Peña and Griffiths (2009) also showed that the probiotic *L. acidophilus* strain La-5 is capable of modifying *E. coli* O157:H7 virulence *in vitro* and *in vivo*. *L. acidophilus* La-5 secretes a molecule(s), which was able to reduce *E. coli* O157:H7 attachment to gastro-intestinal epithelial cells (Medellin-Peña and Griffiths, 2009) and directly inhibit the transcription of *O157:H7* genes involved in colonization (Medellin-Peña *et al.*, 2007). From the above studies, the potential of probiotics to reduce *E. coli* O157:H7 is promising and many commercial products are currently in use.

Prebiotics

Prebiotics are 'nondigestible food ingredients such as fructooligosaccharides (FOS), inulin and galactooligosaccharides (GOS), that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon' (Gibson and Roberfroid, 1995; Gaggìa *et al.*, 2010). Oligosaccharides are of interest because they are neither hydrolyzed nor absorbed in the upper part of the gastrointestinal tract but stimulate the growth and/or activity of desirable bacteria in the colon (Cummings *et al.*, 2001). The use of prebiotics in cattle has been limited due to the ability of ruminants to degrade most prebiotics, but developments in rumen-protective technologies may allow prebiotics to be used in feedlot and dairy cattle (Callaway *et al.*, 2008b; Doyle and Erickson, 2011). There is a concern that prebiotics may promote satiety and this may decrease feed intake and weight gain in animals (Cani *et al.*, 2005). Ultimately, cattle in feedlots need to be fed energy-dense diets to improve growth and produce a high-quality product and any pathogen reduction benefit from a diet should not come at an increased cost for the farm (Berry and Wells, 2010).

Bacteriophage

Bacteriophages are obligate parasites that prey upon specific host bacteria (Greer, 2005). Phages have narrow target spectra and this allows them to be used as potential alternatives to control selective pathogens in mixed microbial populations (Callaway *et al.*, 2003; LeJeune and Wetzel, 2007; Niu *et al.*, 2009a). Studies to date suggest that multiple bacteriophages administered in combination are more effective for eliminating *E. coli* O157:H7 (Bach *et al.*, 2002; O'Flynn *et al.*, 2004; Niu *et al.*, 2009b). A mixture of three O157-specific bacteriophages

was shown to lyse liquid cultures of *E. coli* O157:H7 at 4 and 37°C. However, no individual phage was able to eliminate the culture (Kudva *et al.*, 1999). O'Flynn *et al.* (2004) also found that a mixture of bacteriophages reduced the numbers of *E. coli* O157:H7 in *in vitro* challenge tests. Similarly, in an *in vivo* study conducted by Callaway *et al.* (2008c), the authors found that a cocktail of phages isolated from cattle feces reduced *E. coli* O157:H7 populations in the feces of sheep by 24 h after phage treatment.

Grauke *et al.* (2002) identified the rectoanal junction of cattle as the predominant site for colonization by *E. coli* O157:H7. Hence, Sheng *et al.* (2006) applied a combination of two phage strains directly to the rectoanal junction site of the cattle. In addition, the researchers also continuously administered bacteriophage (10^6 plaque-forming units (PFU) ml^{-1}) orally via drinking water. Both treatments reduced but did not eliminate *E. coli* O157:H7 in the inoculated steers. In another study, Rozema *et al.* (2009) demonstrated that the oral administration of bacteriophage resulted in a lower level shedding of *E. coli* O157:H7 compared to rectal application. This may be due to the increased retention period within the digestive tract, which allows phages to replicate. Acid resistance of phage is critical in oral application to ensure that a sufficient amount of active phages reach the large intestine (Dini and de Urraza, 2010). Stanford *et al.* (2010) developed polymer-encapsulated phages (Ephage) in combination with bolus or feed delivery systems. Ephage successfully released active phages when the capsule reached the large intestine and the pH was more than 7.0. However, Ephage did not reduce the shedding of *E. coli* O157:H7 in the treated steers.

Bacteriophage therapy appears to be most effective when *E. coli* O157:H7 populations are $\geq 10^4$ CFU g^{-1}. Hence, this method can be a strategic intervention option targeted at super shedders within a herd. Hide contamination and transmission can be reduced if fecal concentrations of *E. coli* O157:H7 are kept below 200 CFU g^{-1} (Arthur *et al.*, 2009). This can subsequently reduce the contamination load at the slaughter facilities (Rozema *et al.*, 2009). However, there are still issues of gaining regulatory approval, development of phage resistance and the possibility of genetic materials being transferred to bacterial hosts (Joerger, 2003). In order to obtain the necessary regulatory approval for bacteriophage therapy, Bach *et al.* (2002) suggested sequencing the genome of bacteriophages, demonstrating that undesirable genes are not transferred from bacteriophage to non-target bacteria, and evaluating the effects of the bacteriophage on *E. coli* O157:H7 toxin production.

Vaccination

E. coli O157:H7 infection in cattle requires type-III secreted proteins (TTSP) which enable the bacteria to colonize the intestinal and recto-anal junction mucosa. Hence, a vaccine based on type-III secreted proteins of *E. coli* O157:H7 was developed by Potter *et al.* (2004). The vaccine reduced the prevalence, duration and magnitude of *E. coli* O157:H7 fecal shedding in experimentally inoculated cattle. However, in early studies the vaccine did not significantly reduce the prevalence of fecal *E. coli* O157:H7 in feedlot cattle when tested in nine feedlots under commercial conditions (Van Donkersgoed *et al.*, 2005). After subsequent reformulations of the vaccine (e.g. using different dosage and adjuvant), the vaccine product effectively reduced the colonization of cattle by *E. coli* O157:H7 (Peterson *et al.*, 2007c; Smith *et al.*, 2009). A two-dose vaccination regime resulted in feedlot cattle being 98.3% less likely to have their terminal rectal mucosa colonized by *E. coli* O157:H7 (Peterson *et al.*, 2007b). Another study by Rogan *et al.* (2009) showed that vaccination with the *E. coli* O157:H7 Type-III secretion proteins vaccine decreased the environmental pen-level prevalence of *E. coli* O157:H7. Similarly, Peterson *et al.* (2007c) found that vaccinating a majority of cattle within a pen reduced bacterial shedding among unvaccinated cattle sharing the pen.

Another development in vaccination against *E. coli* O157:H7 is the use of siderophore receptor and porin (SRP) proteins. The SRP protein vaccine reduced the burden of *E. coli* O157:H7 in cattle by targeting the SRP proteins of *E. coli* to disrupt their iron transport system (Fox *et al.*, 2009; Thomson *et al.*, 2009). The vaccine reduced fecal prevalence and fecal concentration of *E. coli* O157:H7. A three-dose vaccine resulted in a two-log reduction of *E. coli* O157:H7 in feces (Thomson *et al.*, 2009).

Husbandry

In the UK, cattle are graded before slaughter according to a five-point cleanliness scoring system. This is in accordance with the Meat Hygiene Service's Clean Livestock Policy with lower scores of 1–2 given to clean and dry animals and scores of 4–5 given to filthy and wet animals. Only livestock classed as categories 1 and 2 (clean and dry/slightly dirty and dry/damp are allowed to proceed to slaughter without further interventions (FSA, 2007)). This underscores the importance of hide cleanliness which helps to minimize the transfer of pathogens to the carcass during dressing. Providing sufficient clean and dry bedding will be the most effective means of preventing heavy soiling of the brisket area (Reid *et al.*, 2002). In another study, the housing of cattle on pens surfaced with pond ash (a by-product from coal combustion) or pens surfaced with soil did not affect fecal shedding of *E. coli* O157:H7 by cattle (Berry *et al.*, 2010). An adequate design and layout of the resting and feeding area and the use of scrapers are also important hygienic measures. However, Barker *et al.* (2007) reported that

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even though automatic scrapers can improve hygiene in barns because of frequent scraping, they can also make cattle dirtier because of the wave of slurry that coats the claws and possibly the lower legs of cattle. The surface properties and cleanability of flooring and feeding surfaces of cattle pens may also affect food safety (Kymäläinen *et al.*, 2009; Määttä *et al.*, 2009). For example, coatings were found to improve cleanability of concrete (Kymäläinen *et al.*, 2008).

Providing clean, dry bedding and maintaining animals in the same group showed a 48% reduction in *E. coli* O157:H7 burden over 4.5 months compared to 18% on the control farms (Ellis-Iversen *et al.*, 2008). This study was in agreement with one by Ward *et al.* (2002) who showed that wet and dirty bedding with temperatures ranging from 15 to 45°C was conducive for *E. coli* growth (up to 10^6 CFU g^{-1}). In addition to dry and clean bedding, the quantity of straw, diets that produce firmer feces, and stocking density are possible factors contributing to the cleanliness of the bedding. Indoor housing was also associated with a higher risk (Ellis-Iversen *et al.*, 2009). The exclusion of wild animals from livestock is beneficial since it is possible that *E. coli* O157:H7 may be introduced into cattle populations through the environment, feed and water contaminated with wild animals' feces (Daniels *et al.*, 2003; Synge *et al.*, 2003; Wetzel and LeJeune, 2006).

Training and motivation as preventive control

The training and education of farmers should be considered as a primary preventive control. Training of farmers in farm food safety risk assessment could encourage the farmers to identify and control potential food safety hazards on farms (Soon *et al.*, in press). Ellis-Iversen *et al.* (2010) interviewed 43 cattle farmers from England and Wales and found that none of the farmers had implemented zoonotic control programs in their farm and less than 50% had an intention to do so. Although the farmers projected positive attitudes towards providing safe products, this study indicated that intention was often hindered by a lack of belief in self-efficacy. One way of promoting adoption of zoonotic control programs would be to simplify the advice on how to control several zoonotic agents. The ability to reduce or control multiple zoonotic agents using a few measures may appeal to farmers, hence may increase rate of adoption (Ellis-Iversen *et al.*, 2010).

Conclusion

In order to be acceptable, control measures need to provide significant reduction in carriage and shedding of *E. coli* O157:H7 and to be low cost. The majority of meatborne *E. coli* O157:H7 outbreaks can be traced back to production on the farm, highlighting the importance of trying to reduce carriage and shedding of this bacterium by cattle at the farm. The number of cases of *E. coli* O157:H7 infection in the US decreased to <1/100,000 persons by 2010 and the percentage attributable to contaminated beef is declining, but interventions at the farm have the potential to dramatically further reduce these numbers. Numerous interventions that could be applied at the farm level have been investigated over the past 20 years, but most have not been shown to be effective and practical.

Management measures such as provision of clean, dry bedding (Ellis-Iversen *et al.*, 2007, 2008) and other steps that minimize fecal–oral spread of the bacterium in the herd appear to be able to reduce the prevalence of *E. coli* O157:H7 in cattle. The administration of *L. acidophilus* NP-51 appears promising (Brashears *et al.*, 2003a; Younts-Dahl *et al.*, 2004; Loneragan and Brashears, 2005; Peterson *et al.*, 2007a) and many probiotic preparations are in commercial production. Vaccines are also available and appear to reduce the shedding of *E. coli* O157:H7 by cattle but there may be issues associated with unrecovered cost to the farmers. There is good evidence that supershedders are an important target and efforts to simply identify these cattle may yield substantial benefits.

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