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Escherichia coli, cattle and the propagation of disease

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One sentence summary: Understanding factors that shape *Escherichia coli* shedding by cattle promises to advance prophylactic and therapeutic interventions and has critical medical and public health implications.
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ABSTRACT

Several early models describing host–pathogen interaction have assumed that each individual host has approximately the same likelihood of becoming infected or of infecting others. More recently, a concept that has been increasingly emphasized in many studies is that for many infectious diseases, transmission is not homogeneous but highly skewed at the level of populations. In what became known as the '20/80 rule', about 20% of the hosts in a population were found to contribute to about 80% of the transmission potential. These heterogeneities have been described for the interaction between many microorganisms and their human or animal hosts. Several epidemiological studies have reported transmission heterogeneities for *Escherichia coli* by cattle, a phenomenon with far-reaching agricultural, medical and public health implications. Focusing on *E. coli* as a case study, this paper will describe super-spreading and super-shedding by cattle, review the main factors that shape these transmission heterogeneities and examine the interface with human health. *Escherichia coli* super-shedding and super-spreading by cattle are shaped by microorganism-specific, cattle-specific and environmental factors. Understanding the factors that shape heterogeneities in *E. coli* dispersion by cattle and the implications for human health represent key components that are critical for targeted infection control initiatives.

Keywords: *Escherichia coli*; super-shedding; super-spreading; virulence; cattle

SUPER-SPREADING IN INFECTIOUS DISEASES

While the term super-shedding and super-spreading have been used interchangeably in the literature, the two are not identical. It was proposed that the term super-shedder should be used to describe a host that for a certain length of time disperses many more microorganisms of a certain type than most other hosts in the same population, while the term super-spreader should be used to refer to a host that presents many more opportunities than most other hosts from the same population to disperse a given pathogen (Chase-Topping *et al.* 2008). Thus, while super-shedding mostly describes the host–pathogen relationship, super-spreading essentially portrays the interaction among different hosts within the same population (Chase-Topping *et al.* 2008). While sometimes a super-shedder may also be a super-spreader, it is important to remark that this is not necessarily a requirement. Super-spreading events have been described in the transmission of several pathogens, including MERS coronavirus (Hui 2016), Ebola virus (Agua-Agum *et al.* 2016), SARS coronavirus (Cori *et al.* 2009; Wong *et al.* 2015), Lassa virus (Lo Iacono *et al.* 2015), influenza virus (Pestre *et al.* 2012), *Coxiella burnetti* (Porten *et al.* 2006), West Nile virus (Kilpatrick *et al.* 2006) and *Mycobacterium tuberculosis* (Jones *et al.* 2009).

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EPIDEMIOLOGY OF *ESCHERICHIA COLI* SHEDDING BY CATTLE

The first time when *E. coli* O157:H7 was linked to human outbreaks was in 1982, when two outbreaks of gastrointestinal illness caused by undercooked meat were reported and, prior to that, the same serotype was isolated in 1975 from a California woman who presented a self-limited gastrointestinal illness (Riley *et al.* 1983). Since then, the bacterium became distributed globally, causing outbreaks, most of them community-acquired and transmitted by the foodborne and waterborne routes (Riley 2014; Munns *et al.* 2015; Kim *et al.* 2016). *Escherichia coli* O157 and *E. coli* O157:H7 may be acquired by person-to-person transmission (Spika *et al.* 1986), the foodborne and waterborne routes, through farm visits and animal contact (Rangel *et al.* 2005; Money *et al.* 2010), from improperly chlorinated swimming pools (Friedman *et al.* 1999) and from exposure while visiting petting zoos (Goode *et al.* 2009). The bacteria have been found in air droplets from commercial beef processing plants and could possibly be transmitted by the airborne route (Varma *et al.* 2003; Schmidt *et al.* 2012). *Escherichia coli* O157:H7 has a very low infectious dose, of only ~50 CFU (Lim, Yoon and Hovde 2010).

Escherichia coli O157:H7-caused human illness can exhibit a variety of clinical manifestations. Some infected individuals may remain asymptomatic, while others can present intestinal and extraintestinal manifestations of varying degrees of severity. These include fever, vomiting, abdominal cramps with watery diarrhea, diarrhea with the presence of blood in the stools, ranging from bloody streaks to visible blood, or hemolytic uremic syndrome and thrombotic thrombocytopenic purpura, which are two of the life-threatening complications (Griffin *et al.* 1988; Rowe 1995; Su and Brandt 1995; Wong *et al.* 2000). The infection may also be fatal (Tarr 1995). The incubation time between the ingestion of the bacteria and the onset of diarrhea is usually 3 days but may vary from 2 to 12 days (Page and Liles 2013).

Healthy colonized cattle and other ruminants are the most significant animal reservoir harboring *E. coli* O157:H7 (Ferens and Hovde 2011; Munns *et al.* 2015), and studies have linked ~75% of the human *E. coli* O157:H7 outbreaks to food products of bovine origin (Callaway *et al.* 2009). Other reservoirs that may impact transmission include sheep (La Ragione *et al.* 2012; Soderlund *et al.* 2012; Gencay 2014) goats (Pao *et al.* 2005; La Ragione *et al.* 2009; Mersha *et al.* 2010; Alvarez-Suarez *et al.* 2016; Swift *et al.* 2017), flies (Alam and Zurek 2004; Pava-Ripoll *et al.* 2015; Burrus *et al.* 2016), deer (Renter *et al.* 2001) and avian species (Wetzel and LeJeune 2006; Callaway, Edrington and Nisbet 2014a). An analysis of 390 outbreaks caused by *E. coli* O157 that were reported in the USA between 2003 and 2012 found that 65% of the transmissions involved food, followed by animal contact, person-to-person transmission and the waterborne route (Heiman *et al.* 2015). Cattle generally remain asymptomatic but they may shed the bacteria into the environment (Gansheroff and O'Brien 2000; Pruimboom-Brees *et al.* 2000; Lim, Yoon and Hovde 2010; Nguyen and Sperandio 2012). Among cattle, shedding occurs intermittently (Hancock *et al.* 1997; Kulow *et al.* 2012; Sharma *et al.* 2012), and it was reported that, at any time, up to 50% of the healthy animals excrete *E. coli* O157:H7 in their stool (Lim, Yoon and Hovde 2010). Large variations have been described in the shedding patterns of individual animals, the proportion of shedding animals on farms that harbor them and, over time, in the amount of shedding on the same farm (Smith, Paiba and Ellis-Iversen 2010).

The scientific literature in the field provides evidence for two somewhat distinct, yet at the same time non-mutually exclusive models for super-shedding: the '20/80 rule', which proposes that ~20% of the animals shed about 80% of the bacteria (Omisakin *et al.* 2003; Matthews *et al.* 2013), and the view that all cattle are super-shedders for a short time (Robinson *et al.* 2009; Lammers *et al.* 2016; Munns *et al.* 2016).

When the shedding of groups of cattle is quantitated, a small percentage of the animals that shed *E. coli* O157:H7 appear to disperse most of the bacteria (Omisakin *et al.* 2003; Matthews *et al.* 2006a,b, 2009; Chase-Topping *et al.* 2007; Nguyen and Sperandio 2012). A study that examined the contribution of different cattle shedding intensity groups to the total number of *E. coli* O157 that they shed revealed that even though 86% of the samples contained <1000 CFU/g of feces, they accounted for only <1% of the total number of bacteria that were shed. This indicated that even though super-shedding events are somewhat rare, they dominate the environmental contamination with bacteria (Matthews *et al.* 2013). In a study conducted at a Scottish abattoir, in which fecal material samples were obtained weekly from the animals between May and July 2002, 7.5% of the individual animals that were examined harbored *E. coli* O157 in their feces. Among the *E. coli* O157 carriers, 9% were high shedders, which was defined as shedding $>10^4$ CFU/g, and these animals were accountable for >96% of the bacteria that were shed by all the animals that had been tested (Omisakin *et al.* 2003). These findings underscored that the high shedding that can be seen in some animals could be more important than the prevalence of colonization in the entire cattle population (Omisakin *et al.* 2003). In a cross-sectional study conducted between March 1998 and May 2000 on beef cattle from randomly selected Scottish farms, no *E. coli* O157 shedding was apparent on 78% of the farms, but a very high prevalence of shedding was present on 2% of the farms, on which >90% of the fecal pats that had been sampled contained the bacteria (Matthews *et al.* 2006b). A second analysis, in which fecal pat samples were collected at 1-month intervals during a 1-year period from several farms, revealed that shedding was never seen on some of the farms, and on some other farms it occurred occasionally and for short periods, but a few farms exhibited intense shedding (Synge *et al.* 2003; Matthews *et al.* 2006b). Results from both datasets pointed towards a model in which this highly heterogeneous pattern of shedding was best supported by variability occurring primarily within, as opposed to between the animal farms (Matthews *et al.* 2006b).

Two short-term studies, conducted in June 2002 and in February 2003, examined the elimination of *E. coli* O157 in the feces of calves that had become naturally colonized with the bacteria. Both studies identified highly shedding animals ($>10^3$ CFU/g), but the bacteria were isolated only intermittently from most of the animals examined. The maximum length of time for which an animal shed the bacteria continuously was 4 days in the first, and 15 days in the second one of these studies. These findings point towards the need to develop sampling protocols that would capture the variability in shedding over time (Robinson *et al.* 2004). A study that proposed to examine *E. coli* O157 shedding in the fecal samples collected from two cohorts of naturally infected weaned calves used a combination between two different approaches, the quantitation of bacterial colony-forming units in the animal feces and immunomagnetic separation (Robinson *et al.* 2009). This study revealed that individual cattle presented brief periods of increased-intensity shedding and, over time, the variation in shedding was larger within than between animals (Robinson *et al.* 2009). This study highlighted that unequivocally categorizing animals as being super-shedders may be challenging, due to the fact that shedding may be highly variable even in the same animal over time (Robinson *et al.* 2009).

Super-shedding has far-reaching and actionable practical implications. A cross-sectional study of Scottish cattle farms found that about 20% of the most infectious animals were responsible for about 80% of the transmission events. This study suggested that successful interventions might only have to target animals that exhibit high bacterial carriage, such as 10^4 to 10^5 CFU/g, and that interventions that target animals with lower levels of bacterial carriage, without successfully targeting highly-shedding animals, would most likely fail (Matthews *et al.* 2006a). In an analysis that modeled the relative importance of the inter-host variability in shaping the bacterial load predicted that if prophylactic interventions were targeted towards the 5% of the most infectious animals, it would be possible to reduce the basic reproduction ratio within herds to <1 (Matthews *et al.* 2006a). A study that sampled 24 beef cattle from a commercial herd on an Australian farm in February 2014 found that individual cattle exhibited diurnal variations in their shedding of *E. coli* O157 that occurred suddenly. If samplings had occurred more rarely, such as at >7-day intervals, this variability in shedding could not have been captured, once again underscoring one of the challenges in studying and understanding the dynamics of *E. coli* shedding (Lammers *et al.* 2016).

INSIGHTS INTO VIRULENCE

A variety of factors that influence the dispersion of *Escherichia coli* by bovine species have been described, and they can be grouped into three broad categories, pertaining to the bacterial strain, the animal host and the environment (Munns *et al.* 2015). Some of these factors may be included into more than one of these categories.

Even after being exposed to as many as 10^{10} CFU *E. coli* O157:H7, cattle remain asymptomatic (Baines, Lee and McAllister 2008). In the cattle gastrointestinal tract, *E. coli* O157:H7 is more often found in the hindgut as compared to the animals' rumen, and one of the main colonization sites is the distal part of the rectum (Naylor *et al.* 2003; Walker *et al.* 2010). A study that explored the patterns of gastrointestinal localization and fecal excretion of the bacteria in calves that had been experimentally administered *E. coli* O157:H7 by the oral route reported that in all the animals that became persistently colonized, most bacteria were found within 3–5 cm proximally from the recto-anal junction. In this region, the bovine terminal rectum contains a dense population of lymphoid follicles that are covered by a layer of epithelial cells, a structure known as the follicle-associated epithelium (Naylor *et al.* 2003; Kudva and Dean-Nystrom 2011). Tropism for this region was seen in orally inoculated animals, in animals colonized as a result of their co-habitation with another super-shedding animal and also naturally, as illustrated by the example of a super-shedding animal that was described on a commercial farm (Naylor *et al.* 2003). Moreover, due to the fact that several different *E. coli* O157:H7 strains exhibited this tropism, it was concluded that this characteristic is intrinsic to the O157:H7 serotype, and it does not represent a strain-specific feature (Naylor *et al.* 2003). *Escherichia coli* that did not belong to the O157:H7 serotype appeared to be distributed throughout the intestinal tract, and their largest numbers were found in the large intestine. However, they were not present in larger numbers at the cattle recto-anal junction, an observation that suggested that *E. coli* O157:H7 and the more abundant non-O157:H7 *E. coli* strains differ with respect to their tropism for this region (Naylor *et al.* 2003; Cobbold *et al.* 2007; Lim *et al.* 2007).

The development of actionable interventions based on the colonization with *E. coli* O157:H7 at the bovine recto-anal junction is challenging, and some studies reported that attempts to eliminate the bacteria from this site were not always successful. A study that used a four-strain O157-specific bacteriophage cocktail examined its ability to mitigate the fecal dispersion of *E. coli* O157 by experimentally inoculated steers. The overall mean fecal shedding was higher in steers treated rectally than in steers treated orally and in steers treated both orally and rectally (Rozema *et al.* 2009). Another study that examined steers with long-term *E. coli* O157:H7 carriage reported that when bacteriophages were administered to the recto-anal junction, the number of bacterial colony-forming units decreased, but in most of the animals in the study, the intervention did not clear the bacteria from this location (Sheng *et al.* 2006a). Another research study reported that not all *E. coli* O157:H7 could be killed by topically applied antimicrobials (Naylor *et al.* 2007). One way to reconcile these findings is to consider the finding that epithelial cells located at the recto-anal junction of the bovine species internalize a subpopulation of the bacteria (Sheng *et al.* 2011).

Escherichia coli O157:H7 is a human pathogen that belongs to the Shiga toxin-producing *E. coli* or STEC group, an autonomous and serologically diverse group of bacteria that carry Shiga toxin-encoding genes on bacteriophages (Beutin *et al.* 2004; Venegas-Vargas *et al.* 2016). These strains synthesize Shiga toxin 1 (Stx1), Shiga toxin 2 (Stx2) or both (Obrig *et al.* 2003; Rahal *et al.* 2012), and additional toxin subtypes have also been described (Scheutz *et al.* 2012). In humans, vascular damage caused by the Stx toxins in the colon and the kidneys was implicated in two major complications: hemorrhagic colitis and the hemolytic uremic syndrome (Richardson *et al.* 1988; Kelly *et al.* 1990; Pruimboom-Brees *et al.* 2000). *Escherichia coli* O157:H7 causes fatal ileocolitis in newborn calves but, as opposed to humans, the calves do not develop extraintestinal vascular lesions (Pruimboom-Brees *et al.* 2000; Kolenda, Burdukiewicz and Schierack 2015). Among cattle, most infected animals remain disease-free for most of their lives (Pruimboom-Brees *et al.* 2000).

Globotriaosylceramide (Gb_3) is the eukaryotic host cell receptor for Stx1 and Stx2 (Samuel *et al.* 1990; Boyd *et al.* 1993; Pruimboom-Brees *et al.* 2000). Gb_3 is a neutral glycosphingolipid in which a ceramide moiety is bound to the α-gal(1→4)-β-gal(1→4)-β-glc trisaccharide (Lindberg *et al.* 1987; Waddell *et al.* 1988; Obrig 2010; Melton-Celsa 2014). The amount of receptor that is expressed on the surface of a target cell was found to shape the cytotoxic effects caused by the Shiga toxins (Schuller 2011). In the kidney, the Gb_3 receptor is expressed on glomerular endothelial cells, podocytes, mesangial cells and on cells from the proximal tubules (Hughes *et al.* 2000; Obrig 2010). The amount of the Gb_3 receptor that is present on human renal microvascular endothelial cells is about 50 times larger than the amount found in larger blood vessels (Obrig *et al.* 1993; Amaral *et al.* 2013). A key mechanism of action of Stx1 and Stx2 involves their ability to inhibit the synthesis of proteins, but they have additional effects, such as the activation of cellular stress signaling pathways (Jacewicz *et al.* 1999; Lee *et al.* 2016). These molecular effects were linked to necrosis and apoptosis in the cells of the microvascular endothelium (Pruimboom-Brees *et al.* 2000). Stx-mediated vascular damage is thought to lead to hemorrhagic colitis in the human colon and to hemolytic uremic syndrome in kidney cells (Pruimboom-Brees *et al.* 2000). Blood vessels from several bovine tissues examined, including the kidneys

and the gastrointestinal tract, did not exhibit binding to Stx1 or Stx2 (Pruimboom-Brees *et al.* 2000).

Microbial factors involved in super-shedding

Several studies identified *E. coli* O157 phage type 21/28 (PT 21/28) as a pathogen-related factor that increases the shedding of the bacteria. Phage type 32 was significantly less likely to be found in a super-shedder strain (Halliday *et al.* 2006; Chase-Topping *et al.* 2007; Chase-Topping *et al.* 2008). It was suggested that PT 21/28 could represent a genomic marker for certain genetic characteristics of the bacterial strains that harbor this phage (Chase-Topping *et al.* 2008).

The mechanistic basis that explains the association of this phage type with super-shedding is not well understood. One model has proposed that strains harboring PT 21/28 present changes in the way the bacterial type III secretion system (T3SS) is regulated, and that these changes could allow more extensive bacterial colonization and excretion (Chase-Topping *et al.* 2007, 2008). In cattle infected with *E. coli* O157:H7, virulence factors that belong to the T3SS are essential for intestinal colonization and for prolonged bacterial shedding (Roe *et al.* 2004; Naylor *et al.* 2005; Sharma *et al.* 2012). Gram-negative bacterial pathogens, as well as many non-pathogenic bacteria, contain type III secretion systems, which are complexes of >20 bacterial proteins that are encoded in a pathogenicity island of the bacterial genome, which is known as the locus of enterocyte effacement (LEE) (Coburn, Sekirov and Finlay 2007; Galan *et al.* 2014). These proteins form needle-like or syringe-like structures that allow the direct entry of bacterial effector proteins into the host cell, allowing them in this way to bypass the extracellular environment (Coburn, Sekirov and Finlay 2007; Sharma *et al.* 2012). Vaccination with a vaccine prepared from a type III secreted protein was able to both decrease the number of shedding animals and reduce the number of bacteria shed by individual animals, a finding that points towards the promise of this intervention (Allen *et al.* 2011).

PT 21/28 is also clinically relevant. In a study conducted in the United Kingdom and Ireland, children with *E. coli* O157 infections with strains harboring PT 21/28 were at a significantly higher risk to develop diarrheal disease-associated hemolytic uremic syndrome than children who were infected with *E. coli* O157 species harboring other phage types (Lynn *et al.* 2005). A national study conducted in Scotland reported that PT 21/28 is the most frequent phage type that has been reported in cattle and in human patients (Pearce *et al.* 2009).

In a study that proposed to examine whether the *E. coli* O157:H7 strain type is connected to super-shedding, investigators collected fecal swabs from about 3500 cattle during the summer months of 2009 and 2010 (Arthur *et al.* 2013). Super-shedding animals represented about 2% of the cattle population tested. The 102 strains obtained from super-shedders represented 52 distinct PFGE genotypes, which were categorized into 19 different phage types, and the genotypes isolated from super-shedding animals did not show any clustering (Arthur *et al.* 2013). This study revealed that in *E. coli* super-shedder strains, the T nucleotide was more frequently present at position 255 of the *tir* gene than the A nucleotide (71% vs 29%) (Arthur *et al.* 2013). Previously, the T255A *tir* allele at this position was found in >99% of 108 human clinical isolates examined and in 55% of 77 bovine isolates, suggesting that it could provide a bovine ecological niche for the bacteria (Bono *et al.* 2007). The *tir* gene encodes a bacterial protein, Tir (translocated intimin receptor), which is essential for colonizing the terminal rectal mucosa of cattle (Sheng *et al.* 2006b). Tir and intimin are encoded by the LEE5 operon (Sheng *et al.* 2006b). Tir, a protein that is produced by the bacterial cell and subsequently inserted in the plasma membrane of the eukaryotic target cell, is the receptor for intimin, which is a virulence factor produced by the bacterium. The binding between the two proteins appears to provide the primary mechanism by which attachment/effacement pathogens are able to intimately adhere to the enterocyte surface (Kenny *et al.* 1997; McWilliams and Torres 2014; Franzin and Sircili 2015). This system represents an instance in which a bacterium synthesizes the receptor to which it will later adhere, after inserting it into the surface of its mammalian target cell (Kenny *et al.* 1997).

Further insight into the genetic factors involved in super-shedding was provided by a study that sequenced the super-shedding *E. coli* O157:H7 strain SS17 that had been isolated from cattle in the midwestern USA and performed a comparative analysis between its genome and that of several other O157:H7 strains (Cote *et al.* 2015). This approach identified ~60 genomic targets that were assessed for their potential contribution to super-shedding, and several non-synonymous SNPs in virulence and adherence genes attracted particular interest. Most non-synonymous polymorphisms were found in genes encoding non-fimbrial adhesins, including *cah*, *yfaL* and *toxB*. The *cah* gene encodes a protein thought to have functions related to adherence, autoaggregation and biofilm formation (Torres *et al.* 2002). The polymorphism in the *cah* gene introduced a non-sense mutation that resulted in a 170-amino-acid truncation of the protein (Cote *et al.* 2015). The *yfaL* gene encodes an adhesin that is overexpressed in uropathogenic *E. coli* strains (Berry, Klumpp and Schaeffer 2009) and increases *in vitro* biofilm formation and the adherence of the bacteria to abiotic surfaces (Roux, Beloin and Ghigo 2005; Chauhan *et al.* 2013). The *toxB* gene is involved in adherence to epithelial cells (Cote *et al.* 2015). This study also examined polymorphisms in the upstream 250 base-pair region of 108 genes and in the downstream 100 base-pair region of 95 genes as compared to the reference genomes. It is thought that changes in these regions, which contain gene promoter and terminator sequences, could shape super-shedding by altering protein expression (Cote *et al.* 2015).

The SS17 strain showed a unique ability to adhere to squamous epithelial cells located at the bovine recto-anal junction, and all cells exposed to this strain had >10 adhering bacteria per cell, significantly more than in the case of two other *E. coli* O157 isolates. Anti-sera against several LEE-encoded proteins, including Tir and intimin, decreased negligibly SS17 adherence to squamous epithelial cells located at the bovine recto-anal junction, but blocked their adherence to human HEp-2 cells, indicating that SS17 uses adherence mechanisms that are distinct from, or independent of these LEE-encoded proteins (Cote *et al.* 2015). A subsequent effort led to the sequencing of a second super-shedder strain, SS52 (Katani *et al.* 2015). This strain contained 3106 SNPs when compared to EDL933, which is a strain isolated from Michigan ground beef that has been used as a reference strain for the O157:H7 serotype, and 801 SNPs when compared to the SS17 genome (Katani *et al.* 2015). Further analyses of these SNPs may provide insight into some of the genetic bases of super-shedding.

Cattle-specific factors involved in super-shedding

Several cattle-specific factors suspected to shape the duration of *E. coli* O157 shedding by the animals have been examined. One of these factors is the proliferation rate of the cells from the cattle gastrointestinal tract. Faster proliferation of the cells

in the large intestine increased the likelihood that the lower gastrointestinal tract of the animals will remain colonized for a significantly longer time as compared to animals that had slower cellular proliferation rates (Magnuson *et al.* 2000). Animal transportation is another factor that was shown in some studies to increase the shedding of bacteria, including *E. coli* O157:H7, by cattle and other animal species (McCluskey *et al.* 1999; Barham *et al.* 2002; Bach *et al.* 2004; Berry and Wells 2010). Dietary stress and food deprivation in transported cattle, which may occur when food is not available or when animals refuse to eat, also increase the shedding of bacteria (Cray *et al.* 1998). A study that inoculated calves with 10^7 *E. coli* O157:H7 following a 48-h period of food deprivation reported that susceptibility to infection and the shedding of bacteria were higher in the food-deprived animals, as compared to animals that had been administered a regular diet (Cray *et al.* 1998). Another study that examined the link between animal feed and gut bacteria found that cattle that had been fasted for 24 h before transportation had more *E. coli* in the digesta from several sites, including their rumen, small intestine and large intestine, as compared to animals that had been pasture-fed or hay-fed for 48 h prior to transportation (Gregory *et al.* 2000).

Studies that examined the presence of STEC in beef cattle found that animal age is one of the factors involved in super-shedding. Heifers (animals at least 1 year of age) had a significantly lower prevalence and lower shedding than cows (animals at least 2 years old with at least one calf) but higher concentrations of bacteria, when these were present. The prevalence of STEC was highest in 2-year-old cows, and it showed a decrease in older animals (Mir *et al.* 2015). Higher STEC prevalence and shedding were associated with younger cattle age in beef cattle (Cray and Moon 1995; Zhao *et al.* 2013). A study in dairy cattle reported that the shedding of VTEC was highest in 2- to 6-month-old calves as compared to calves younger than 2 months old and older than 6 months old and cows (Nielsen *et al.* 2002).

Some studies did not find the sex of the calf to be significantly associated with shedding (Mir *et al.* 2015, 2016), others found that shedding was more intense in herds having many bull calves (Nielsen *et al.* 2002) and other authors reported that higher *E. coli* shedding can be seen on farms that harbor female breeding cattle (Chase-Topping *et al.* 2007). In one study, castration decreased the super-shedding of *E. coli* O157, which was significantly higher in bulls as compared to steers, despite the fact that the prevalence of the bacterium was comparable between the two animal groups (Jeon *et al.* 2013). A study that examined dairy cattle farms from Minnesota, in a search for herd-specific risk factors associated with the shedding of bacteria, found that bacteria encoding Shiga toxins were more often shed in animals from small herds, harboring fewer than 100 animals, than in large herds that contained 100 or more animals (Cho *et al.* 2013).

A study that sought to identify host factors that could account for super-shedding performed RNA sequencing (RNA-Seq) to compare transcriptomic profiles of the rectal tissue in super-shedding and non-super-shedding cattle. This approach identified 58 differentially expressed genes and, among these, 31 genes that were downregulated in super-shedders had functions linked to innate and adaptive immunity. The analysis of these genes pointed towards a decrease in the number and a decline in the function of several types of immune cells in super-shedding animals. These observations pointed towards the possibility that an impairment in the ability to mount an immune response and immune suppression caused by the bacterial colonization in super-shedding animals are potential factors that could be involved (Wang *et al.* 2016).

Diet composition emerges as a critical factor that influences super-shedding. However, many of the studies that examined the link between diet and super-shedding did not show clear or consistent trends. The interpretation of most of these studies is challenging due to the fact that for every study that reported increased shedding after exposure to a specific dietary feed component, there are usually other studies that showed no impact or possibly reduced shedding. Several years ago, a study revealed that cattle fed with grain had larger numbers and more acid-resistant *E. coli* in their colon than animals that had been fed with hay. In cattle fed hay and in cattle at pasture (fed with fresh grass), the colon pH was >7, the animals had ~20 000 *E. coli* per gram of colon contents, and almost all these bacteria were killed by high acidity. In animals fed a diet in which 60% of the dry matter consisted of grain, the colon pH decreased to 6.9 (a decrease that was not statistically significant) and 6.3×10^6 viable *E. coli* cells per gram could be recovered from the colon. This bacterial population contained >25 000 acid-resistant viable *E. coli* per gram of colon material. In animals fed a diet in which >80% of the dry matter consisted of grain, the colon pH decreased to 5.9 (a statistically significant change) and 250 000 viable, acid-resistant *E. coli* were found per gram of colonic digesta (Diez-Gonzalez *et al.* 1998). Additional controlled experiments showed that in cattle fed hay, volatile fatty acids, such as acetic, propionic and butyric acid, were present at very low concentrations in the colon, and the administration of animal feeds containing increasing percentages of grain led to an ~4-fold increase in their concentrations in the colon. While hay-fed cattle had about 10^5 coliform bacteria/g of feces in the colon, the number of these bacteria increased 1000-fold in animals fed 90% grain, and most of these bacteria were identified as *E. coli* (Diez-Gonzalez *et al.* 1998). The abrupt shift from hay to a grain diet also increased the acid-resistance of the colonic *E. coli* populations. After animals were switched from the grain to a hay diet, the number of viable *E. coli* cells in the colon decreased, and 5 days later it was almost 10^6-fold lower (Diez-Gonzalez *et al.* 1998).

A study found significantly larger numbers of *E. coli* O157:H7 in cattle that had been fed grain than in cattle that had been fed hay, at both 4°C and 25°C (Lowe *et al.* 2010). In another study, the duration of shedding was compared in healthy Holstein steers that were fed hay or grain, after they had been experimentally inoculated with *E. coli* O157:H7. Shedding was seen for a longer time in animals fed hay (grass or alfalfa) (39–42 days on average) than in those fed grain (4 days on average), and the bacteria isolated from animals in the two groups did not show any differences in their acid resistance (Hovde *et al.* 1999).

To examine the link between the intestinal microbiota and super-shedding, a study sequenced the ribosomal genes from the fecal microbiota of 11 *E. coli* O157:H7 super-shedders and 11 non-shedding animals from a commercial setting in Canada and interrogated the data to identify potential differences between the two groups of animals (Xu *et al.* 2014). From 400 cattle that were sampled, 11.5% shed the bacteria and 2.8% were super-shedders (>10^4 CFU/g of feces). Clustering revealed that the microbiota of super-shedding animals was more rich and diverse as compared to that of non-shedding animals. The study identified 72 operational taxonomical units that were differentially enriched in the microbiota from the two animal groups, and 69 of these belonged to the *Firmicutes* and *Bacteroidetes* phyla (Xu *et al.* 2014). However, it is not yet clear whether super-shedding represents the cause or the effect of these differences in the intestinal microbiota (Munns *et al.* 2015).

The intestinal microbiota of mammals, which was reported to impact host health and physiology, is to a large extent shaped

by diet (Durso *et al.* 2012; Petri *et al.* 2013; Golder *et al.* 2014; Myer *et al.* 2015; Sala *et al.* 2016). One of the dietary supplements that received attention in context of bacterial colonization and shedding by cattle is distillers' grain (Durso *et al.* 2012). Distiller's grain, which can be produced from a variety of grains, including corn, wheat and sorghum, is a by-product of ethanol synthesis during the process of grain-derived starch fermentation, and provides energy and protein sources for cattle (Jacob *et al.* 2008; Schingoethe *et al.* 2009; Durso *et al.* 2012). Non-starch constituents, such as fibers, proteins, and lipids, are concentrated ∼3-fold in distiller's grain (Jacob *et al.* 2008; Klopfenstein, Erickson and Bremer 2008). Distiller's grain has been used for over 100 years, and two varieties, wet and the dry, are available for use in cattle feed (Schingoethe *et al.* 2009; Durso *et al.* 2012). Of the two varieties, the dry one is easier to transport to cattle producers (Jacob *et al.* 2008; Klopfenstein, Erickson and Bremer 2008).

The type of distiller's grain is one of the factors that affect the shedding of bacteria by cattle. Several studies reported that dried distiller's grain prepared from corn increases the fecal shedding of *E. coli* O157:H7 by cattle (Jacob *et al.* 2008; Wells *et al.* 2009; Yang *et al.* 2010), but this relationship is not supported by all the studies (Hallewell *et al.* 2013). In the fecal microbial communities of beef cattle that were fed diets containing 40% wet distiller's grains with solubles (WDGS), several microbial genera were consistently more enriched as compared to the microbiota of animals that had been fed corn (Durso *et al.* 2012). In a study that inoculated calves with *E. coli* O157 orally, and provided feeds with and without corn-derived dried distiller's grain, the concentration of bacteria was higher in the cecum, colon, rectum and feces of the animals that had been supplemented with dried distiller's grains as compared to control animals, and this was accompanied by increased bacterial shedding (Jacob *et al.* 2008). Another study reported that the prevalence of *E. coli* O157:H7 fecal shedding and the number of animals that shed large numbers of bacteria were not different at a statistically significant level between cattle fed with a control diet and cattle fed with a 20% distiller's grain diet, but cattle fed with 40% distiller's grain harbored significantly more highly shedding animals (Jacob *et al.* 2010). In another study, the prevalence of the stool *E. coli* O157:H7 was higher in animals fed a diet in which WDGS was 40% or 70% of the dry weight (35.4% and 38.4%, respectively) than in animals fed a diet without WDGS (1.9%) (Wells *et al.* 2011). When some animals were switched between diets, it was found that switching to lower WDGS levels decreased the number of *E. coli* O157:H7 excreted in the animals' feces after 56 days, but not after 28 days (Wells *et al.* 2011). In another study that proposed to examine the effect of the diet on shedding, animals from a group of 40 heifers that shed *E. coli* O157 were randomly allocated to sorghum or wheat diets, and each of these diets was processed by either steam flaking or dry rolling. The fecal shedding was lower in the animals fed grains prepared by dry rolling as compared to the animals fed grains prepared by steam flaking. The authors hypothesized that in animals fed dry-rolled grains, more digested material could undergo fermentation after entering the hindgut, and this would render the hindgut environment more adverse for bacterial survival (Fox *et al.* 2007).

Various feed additives were also shown to affect the shedding of bacteria by cattle. Supplementing feedlot cattle with the feed additive Tasco-14 (*Ascophyllum nodosum*) led to a decrease in the *E. coli* fecal prevalence (Braden *et al.* 2004). In a study that administered 10^{10} colony-forming units of *E. coli* O157:H7 orally to sheep that were fed, for 10 days, a mixture of dried orange pellet and fresh orange peel that constituted 10% of the dry weight of their feed, the fecal shedding of the bacteria was reduced after 96 h (Callaway *et al.* 2011). The dietary supplementation of Holstein calves with chitosan microparticles significantly decreased the count of *E. coli* O157:H7 that the animals shed, and also reduced the time for which the animals shed the bacteria (Jeong *et al.* 2011). Chitosan microparticles are positively charged, and their ability to bind, *in vitro*, the *E. coli* cells, which have a negatively charged surface, provides a potential mechanism to explain their ability to decrease bacterial shedding (van der Lubben *et al.* 2001; Hamadi *et al.* 2008; Jeong *et al.* 2011). Ractopamine, a synthetic β-adrenergic agonist (Colbert, Williams and Williams 1991), used as feed additive for certain food animals (Edrington *et al.* 2006), increased the number of *E. coli* O157:H7 that the sheep excreted into their feces, and decreased *Salmonella typhimurium* fecal dispersion in pigs (Edrington *et al.* 2006b).

Several studies revealed that the administration of sodium chlorate into the animals' water led to a decrease in the number of *E. coli* O157:H7 that could be detected in the cattle rumen and cecum and decreased their fecal excretion of the bacteria (Callaway *et al.* 2002; Callaway, Edrington and Nisbet 2014b), and also reduced the *E. coli* populations in the intestinal tracts of sheep (Callaway *et al.* 2003) and pigs (Anderson *et al.* 2001).

Environmental factors involved in super-shedding

A study conducted at a beef cattle abattoir from Scotland compared *E. coli* O157 prevalence in fecal samples collected during the colder months, between January and March 2003, and during the warmer months, between May and July 2002. Concomitantly, the data were compared with the weekly rates of human *E. coli* O157 infections that were reported in Scotland between 1998 and 2002 (Ogden, MacRae and Strachan 2004). This study estimated that the shedding by individual cattle was more prevalent during the winter period, between January and March, than during the summer period, between May and July (11.2% vs 7.5%). However, the trend for the human infections was reversed, with the average number of human cases being lower in the 8-week colder period as compared to the 8-week warmer period (13 vs 53). The number of high-shedding cattle, defined as animals that shed $>10^4$ CFU/g, was similar during the two time periods (0.66% vs 0.73%, respectively). However, high-shedding cattle were dispersing, on average, six times more bacteria during the summer than during the winter (1932 vs 330 CFU/g). It was this trend, the one that mirrored the number of reported human infections. These findings indicated that simply measuring the prevalence of cattle shedding, at the individual and at the group level, are insufficient to reflect their potential as reservoirs, if the extent of shedding is not incorporated as well (Ogden, MacRae and Strachan 2004). The more intense *E. coli* O157 shedding by the cattle during the warmer months, combined with the increased human exposure by foodborne and environmental routes, and the higher outside temperature that is more permissive for bacterial growth are thought to be some of the factors that collectively explain the seasonality of human infection in Scotland (Ogden, MacRae and Strachan 2004).

A study that proposed to evaluate the link between day length and the dispersion of *E. coli* O157:H7 by cattle examined data collected in nine research studies that had been conducted in three geographical areas from North America, and found a positive relationship between day length and the prevalence of *E. coli* O157:H7. To better understand this observation, an experiment was subsequently designed to interrogate the causal link between artificial lighting and the presence of *E. coli* O157 among

animals on a commercial farm. This experiment revealed that control animals had a significantly lower fecal prevalence of the bacteria than animals that had been artificially exposed to light (Edrington *et al*. 2006a). Some of these factors, such as lightning, are related to animal stress (Collier, Dahl and VanBaale 2006; Bova *et al*. 2014; Disanto *et al*. 2014).

A study conducted on cattle farms in Scotland reported that animals shedding VTEC O157 were more likely to exist on farms that used slurry as compared to manure (Gunn *et al*. 2007). The presence of super-shedders was less likely on farms where older animals were present. Pasture-raised animals exhibited lower mean levels of shedding as compared to housed animals, and recent changes in housing or in the animal diet amplified this effect (Gunn *et al*. 2007). A longitudinal study conducted between March 2000 and February 2001 on young cattle herds in England and Wales, which examined the shedding of VTEC O157, reported that the likelihood to shed the bacteria was 4.7-fold higher if the cattle were kept in pens than if the animals were housed in groups or raised at pasture (Smith, Pollitt and Paiba 2016). In a study conducted in dairy cattle from Michigan farms, STEC shedding increased 2.5-fold when the average temperature during the 1- to 5-day period preceding sample collection exceeded 28.9°C (Venegas-Vargas *et al*. 2016). Cattle lactating for the first time had a 1.8-fold higher risk to shed STEC as compared to animals that have lactated at least twice, and animals that had been producing milk for less than 31 days had a 3.9-fold higher risk to shed the bacteria as compared to animals that had been producing milk for 31 days or longer (Venegas-Vargas *et al*. 2016).

CONCLUSIONS

Escherichia coli O157:H7 was for the first time reported to cause human outbreaks 35 years ago, in 1982, and prior to that, the same serotype was identified in 1975 in a woman with gastrointestinal illness. Since then, the pathogen has become a major cause of foodborne and waterborne illness worldwide, and a medical and public health challenge. The microorganism is thought to have evolved by the horizontal acquisition of virulence genes that encode Shiga toxins and other virulence factors. Cattle, along with some other ruminant species, are the principal animate reservoir for *E. coli* O157:H7 strains. In these animals, the bacteria may be present in the gastrointestinal tract, particularly the terminal recto-anal junction, without causing clinical disease, and asymptomatic animals may shed them from these locations into the environment. *Escherichia coli* super-shedding and super-spreading by cattle is shaped by a multitude of factors, which can be grouped into pathogen-specific, host-specific and environmental causes. Understanding heterogeneities in *E. coli* shedding and transmission by cattle, and on cattle farms, promises to help develop and implement targeted interventions with applications in agriculture, animal husbandry, food safety and human health.

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