Veterinary Public Health Approach to Managing Pathogenic Verocytotoxigenic Escherichia coli in the Agri-Food Chain

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ABSTRACT Verocytotoxigenic Escherichia coli (VTEC) comprises many diverse serogroups, but seven serogroups, O157, O26, O103, O145, O111, O21, and O45, have been most commonly linked to severe human infections, though illness has also been reported from a range of other VTEC serogroups. This poses challenges in assessing the risk to humans from the diverse range of VTEC strains that may be recovered from animals, the environment, or food. For routine assessment of risk posed by VTEC recovered from the agri-food chain, the concept of seropathotype can be used to rank the human risk potential from a particular VTEC serogroup on the basis of both serotype (top seven serogroups) and the presence of particular virulence genes (vt in combination with eae, or aaiC plus aggR). But for other VTEC serogroups or virulence gene combinations, it is not currently possible to fully assess the risk posed. VTEC is shed in animal feces and can persist in the farm environment for extended periods ranging from several weeks to many months, posing an ongoing reservoir of contamination for grazing animals, water courses, and fresh produce and for people using farmland for recreational purposes. Appropriate handling and treatment of stored animal waste (slurries and manures) will reduce risk from VTEC in the farm environment. Foods of animal origin such as milk and dairy products and meat may be contaminated with VTEC during production and processing, and the pathogen may survive or grow during processing operations, highlighting the need for well-designed and validated Hazard Analysis Critical Control Point management systems. This article focuses on a veterinary public health approach to managing VTEC, highlighting the various routes in the agri-food chain for transmission of human pathogenic VTEC and general approaches to managing the risk.

INTRODUCTION

It is well documented that animals and, in particular, ruminants can carry a range of potentially harmful pathogens, including verocytotoxigenic Escherichia coli (VTEC), in their gastrointestinal tract. VTEC can reportedly survive for several months in the environment, in feces, and in soil, which allows for the recycling of VTEC among food animals and wildlife and prolonged environmental contamination. VTEC contamination of fresh produce may arise from irrigation water, manure or compost applied to soil as a fertilizer, and feces of wildlife or farmed animals. Table 1 summarizes some of the diverse VTEC outbreaks over the past few years (2006–2013). Of significant interest is that apart from the newly emerging vehicles of infection, serogroups other than O157 are increasingly causing outbreaks, many of which have severe outcomes with cases of hemolytic-uremic syndrome (HUS) and fatalities. While

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outbreaks are important and gain notoriety, the contribution of sporadic cases of human VTEC infection cannot be ignored. The data show that although foods of animal origin such as meat and dairy products and fresh produce such as salads and vegetables are well recognized important vectors of infection, there have also been VTEC outbreaks related to direct contact with fecal matter through recreational activities including visiting petting zoos, attending agricultural fairs, and swimming in contaminated water (1). With so many routes of potential transmission, it is clear that the management of this pathogen requires a multidisciplinary approach with cooperation among the disciplines of human medicine, animal and veterinary sciences, and environmental and food scientists. A veterinary public health approach to managing VTEC focuses on collation of data on prevalence of VTEC in different sources, the importance of each source as a reservoir of human pathogen VTEC, and risk factors for transmission to humans.

This article provides an overview of the risks for transmission of human pathogenic VTEC from the various routes of transmission, including animals, the farm environment, and production of milk and meat, and highlights general management approaches to reduce the risk of human exposure from such sources.

**HUMAN PATHOGENIC VTEC**

VTEC strains are a heterogeneous group of *E. coli* serogroups, and not all will cause human illness. The diversity of serogroups and virulence factors among human-disease-causing VTEC strains poses considerable challenges in assessing the risk posed by VTEC recovered from the agri-food chain. The dominant pathogenic VTEC serotype remains *E. coli* O157:H7 (2,3) and has the strongest association with HUS worldwide (4). However, a common pattern being observed in the European Union and the United States (5) is the increasing number of reported cases now attributed to non-O157 serotypes. Approximately half of all confirmed VTEC cases in the European Union are now associated with serogroup O157 (6), and in the United States in the period 2000 to 2010, O157 cases decreased from 0.12 to 0.95/100,000 whereas non-O157 cases increased from 2.17 to 0.95/100,000 whereas non-O157 cases increased from 0.12 to 0.95/100,000 (5). Of the non-O157 cases, six VTEC serogroups are most commonly isolated from patients: O26, O103, O145, O111, O21, and O45. However, it should be noted that not all strains belonging to these serogroups will cause severe illness and that other non-O157 VTEC serogroups also cause illness. Pathogenic VTEC strains are categorized as enterohemorrhagic *E. coli*, and usually in these strains, a large outer membrane protein (94–97 kDa) called intimin mediates the intimate contact between the bacterium and the enterocyte cytoplasmic membrane (attachment) and the destruction of the enterocyte microvilli (effacement). The genetic determinants for this (eae, tir, esc, and sep genes) are grouped together on the chromosome, forming a pathogenicity island called LEE, for locus of enterocyte effacement (7). The eae gene encodes for intimin, which is responsible for the intimate attachment, and at least five different forms (α, β, γ, δ, ε)

### TABLE 1

Selected VTEC outbreaks (2006–2013) highlighting different vehicles of infection and serogroups

<table>
<thead>
<tr>
<th>Country</th>
<th>Reference</th>
<th>Year</th>
<th>Serotype</th>
<th>No. of cases</th>
<th>No. of HUS cases</th>
<th>No. of deaths</th>
<th>Likely sources or mode of transmission</th>
</tr>
</thead>
<tbody>
<tr>
<td>United Kingdom</td>
<td>Lauanders et al., 2013 (142)</td>
<td>2013</td>
<td>O157</td>
<td>19</td>
<td>0</td>
<td>0</td>
<td>Watercress</td>
</tr>
<tr>
<td>Denmark</td>
<td>Soborg et al., 2013 (143)</td>
<td>2012</td>
<td>O157</td>
<td>13</td>
<td>8</td>
<td>0</td>
<td>Ground beef</td>
</tr>
<tr>
<td>United States</td>
<td>Slayton et al., 2013 (144)</td>
<td>2012</td>
<td>O157</td>
<td>58</td>
<td>3</td>
<td>0</td>
<td>Romaine lettuce</td>
</tr>
<tr>
<td>Germany</td>
<td>Karch et al., 2012 (10)</td>
<td>2011</td>
<td>O104</td>
<td>4,321</td>
<td>852</td>
<td>32</td>
<td>Sprouts</td>
</tr>
<tr>
<td>United States</td>
<td>McCollum et al., 2010 (104)</td>
<td>2010</td>
<td>O157</td>
<td>41</td>
<td>1</td>
<td>0</td>
<td>Cheese Gouda</td>
</tr>
<tr>
<td>United States</td>
<td>Taylor et al., 2013 (145)</td>
<td>2010</td>
<td>O145</td>
<td>33</td>
<td>3</td>
<td>0</td>
<td>Romaine lettuce</td>
</tr>
<tr>
<td>Netherlands</td>
<td>Greenland et al., 2009 (146)</td>
<td>2009</td>
<td>O157</td>
<td>20</td>
<td>0</td>
<td>0</td>
<td>Steak tartare</td>
</tr>
<tr>
<td>United States</td>
<td>Neil et al., 2012 (147)</td>
<td>2009</td>
<td>O157</td>
<td>72</td>
<td>10</td>
<td>0</td>
<td>Cookie dough</td>
</tr>
<tr>
<td>United States</td>
<td>CDC, 2010 (148)</td>
<td>2008</td>
<td>O157</td>
<td>99</td>
<td>1</td>
<td>0</td>
<td>Ground beef</td>
</tr>
<tr>
<td>Ireland</td>
<td>O’Sullivan et al., 2008 (89)</td>
<td>2008</td>
<td>O157</td>
<td>148</td>
<td>NA*</td>
<td>NA</td>
<td>Private wells drinking water</td>
</tr>
<tr>
<td>Belgium</td>
<td>De Schrijver et al., 2008 (107)</td>
<td>2008</td>
<td>O26 &amp; O145</td>
<td>12</td>
<td>3</td>
<td>0</td>
<td>Homemade ice cream, feral material from farm</td>
</tr>
<tr>
<td>Netherlands/Iceland</td>
<td>Friesema et al., 2008 (149)</td>
<td>2007</td>
<td>O157</td>
<td>50</td>
<td>0</td>
<td>0</td>
<td>Lettuce</td>
</tr>
<tr>
<td>Denmark</td>
<td>Ethelberg et al., 2009 (150)</td>
<td>2007</td>
<td>O26</td>
<td>20</td>
<td>0</td>
<td>0</td>
<td>Fermented beef sausage</td>
</tr>
<tr>
<td>United States</td>
<td>Wendel et al., 2009 (151); Jay et al., 2007 (69)</td>
<td>2006</td>
<td>O157</td>
<td>199</td>
<td>31</td>
<td>3</td>
<td>Fresh spinach and feral pigs</td>
</tr>
</tbody>
</table>

*Data not available.*
have been reported for VTEC strains (8). The tir gene codes for a type III-secreted translocated intimin receptor (Tir protein). The esp genes code for type III-secreted translocated Esp proteins. There are, however, reports of LEE-negative E. coli O157 clones causing illness in humans (9), indicating that such strains express alternative adherence factors that allow them to colonize the intestinal tract.

In 2011, an outbreak strain that contained a combination of vt genes and virulence factors normally seen in enteroaggregative E. coli (EAEC) emerged as the principal protagonist in a major outbreak in Germany. The outbreak resulted in 4,321 confirmed cases, including 852 cases of HUS, with 54 deaths reported in 14 European Union member states, the United States, and Canada (10). This highly unusual hybrid organism combined the vt genes and the adhesion mechanisms of an EAEC (11) instead of the Tir/intimin and type III secretion and the system usually seen in enterohemorrhagic E. coli. Moreover, the strain also demonstrated extended spectrum beta-lactamase phenotype, thus further underlining the enhanced virulence of the strain and the severity of the outbreak, aided by the specific combination of enhanced adhesion, survival fitness, vt2 production, and antibiotic resistance as a result of the high genome plasticity of this E. coli pathogen. This outbreak clone was a clear example of gene acquisition by means of lateral gene transfer that resulted in an accumulation of synergistic virulence factors. This illustrates that pathotypes of E. coli can have common characteristics that overlap and that they do and have the potential to evolve rather than stand as a fixed archetype (12).

Although no single marker or combination of markers defines “pathogenic” VTEC associated with human disease (13), the presence of vt2- and eae-positive strains is associated with a high risk of more serious illness and an increased risk of HUS (14), but other virulence gene combinations and/or serotypes may also be associated with serious disease in humans, including HUS. Patient-associated factors such as age, immune status, and the antibiotic therapy administered may also influence the likelihood and severity of disease.

The concept of seropathotype has evolved, which ranks the potential for a particular VTEC to cause serious human illness on the basis of both serotype and the presence of particular virulence genes. A recent European Food Safety Authority Scientific Opinion (15) proposed that any isolates of VTEC serogroups O157, O26, O103, O145, O111, or O104 that also have eae (intimin) or aaiC (secreted protein of EAEC) plus aggR (plasmid-encoded regulator) genes should be considered as presenting a potentially high risk for diarrhea and HUS. For any other VTEC serogroups with the same combination of virulence genes, the potential risk is regarded as high for diarrhea, but currently unknown for HUS. While for any serogroups not having this gene combination, the currently available data do not allow any inference regarding potential risks. This concept allows food business operators in Europe to make an assessment of the risk posed by a VTEC isolate recovered from a food, animal, or environmental sample. Nonetheless, to support stakeholders and food business operators, more information is urgently needed on what constitutes a human virulent VTEC, and this will remain an active area of research, and rapidly evolving techniques such as whole genome sequencing will progress this area.

**CARRIAGE OF VTEC IN ANIMALS**

VTEC strains have been isolated from a variety of animal carriers, including cattle, sheep, horses, deer, goats, dogs, geese, pigs, and wild birds. Recent studies on prevalence of VTEC in ruminant animals and meats are reviewed in Table 2. Moreover, feces from cattle, sheep, goats, deer, and rabbits have all been linked to cases of infection (16–20). Transmission to humans can occur as a result of direct contact with VTEC-contaminated fecal material, from handling or petting animals, or by exposure to fecally contaminated mud or vegetation during recreational activities.

**Bovine Carriage of VTEC**

Cattle are recognized as a principal reservoir for VTEC (21). VTEC is generally a transient member of the intestinal microflora and only rarely causes disease in young, weakened calves. Although cattle have been shown to harbor this pathogen on occasion in their rumen, VTEC is found more frequently in the distal portion of the bovine gastrointestinal tract, with the rectal-anal junction identified as the predominant colonization site (22, 23). Rhoades et al. extensively reviewed the prevalence of VTEC in the beef chain and the fecal prevalence of E. coli O157 in cattle and showed it varied from 0 to 48.8% (24–27). In the United States, it is reported that most bovine animals have been exposed to E. coli O157 by the time of weaning (28), and the reported prevalence in cattle ranges from 10 to 28% (29). In the European Union, studies have shown that VTEC prevalence in feces ranged in individual animals from 2.1 to 13.5%, in herds from 6.1 to 16.2%, and in
slaughter batches from 13 to 20.2%, and for O157 this ranged from 0.3 to 2.3%, 1.5 to 13.7%, and 5.5 to 20.2%, respectively (6).

Shedding of VTEC by cattle is generally intermittent, with herd members remaining negative for months, with only a proportion sporadically becoming positive for a few weeks at a time (30, 31). Carriage rates are higher in calves than in adult cattle, and while detected all year-round, carriage rates are subject to seasonal effects, with higher rates reported in spring and summer. Other factors thought to influence carriage rates are the ages of animals and farming and husbandry practices (32).

The number of VTEC (CFU g⁻¹) pathogens being shed in the feces of individual animals is considered important in the context of hide, environmental, and subsequent carcass contamination. The typical pattern of shedding in a herd is sporadic, with intense periods of shedding interspersed with periods of nonshedding (33). Ogden et al. have also reported that concentrations of E. coli O157 being shed in the feces of positive cattle were highest during summer months (34).

### TABLE 2

Selected VTEC prevalence studies (2007–2013) showing pathogenic VTEC prevalence in ruminant animals (cattle and sheep)

<table>
<thead>
<tr>
<th>Sample type (n=sample no.)</th>
<th>Serotype(s)</th>
<th>Country</th>
<th>Prevalence (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattle</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carcass (300)</td>
<td>O157</td>
<td>Spain</td>
<td>14.7%</td>
<td>Ramoneda et al., 2013 (152)</td>
</tr>
<tr>
<td>Feces (1,145)</td>
<td>O157</td>
<td>United States</td>
<td>19.7%</td>
<td>Dargatz et al., 2013 (153)</td>
</tr>
<tr>
<td>Feces (1145)</td>
<td>O45, O103, O121, O145, O26, O111</td>
<td>United States</td>
<td>13.8%, 9.9%, 9.3%, 5.5%, 1.1%, 0.5%</td>
<td>Dargatz et al., 2013</td>
</tr>
<tr>
<td>Feces (250)</td>
<td>O157</td>
<td>Mexico</td>
<td>5.2%</td>
<td>Narvaez-Bravo et al., 2013 (154)</td>
</tr>
<tr>
<td>Hides (250)</td>
<td>O157</td>
<td>Mexico</td>
<td>11.7%</td>
<td>Narvaez-Bravo et al., 2013</td>
</tr>
<tr>
<td>Carcass (250)</td>
<td>O157</td>
<td>Mexico</td>
<td>0.8%</td>
<td>Narvaez-Bravo et al., 2013</td>
</tr>
<tr>
<td>Feces (301)</td>
<td>O157</td>
<td>Ireland</td>
<td>2.7%</td>
<td>Thomas et al., 2012 (159)</td>
</tr>
<tr>
<td>Feces (402)</td>
<td>O26, O103, O145</td>
<td>Ireland</td>
<td>1.5%, 8.5%, 0.7%</td>
<td>Thomas et al., 2012</td>
</tr>
<tr>
<td>Hide (301)</td>
<td>O157</td>
<td>Ireland</td>
<td>18.9%</td>
<td>Thomas et al., 2012</td>
</tr>
<tr>
<td>Hide (402)</td>
<td>O26, O103, O145</td>
<td>Ireland</td>
<td>6%, 27.1%, 2.5%</td>
<td>Thomas et al., 2012</td>
</tr>
<tr>
<td>Carcass (301)</td>
<td>O157</td>
<td>Ireland</td>
<td>0.7%</td>
<td>Thomas et al., 2012</td>
</tr>
<tr>
<td>Carcass (402)</td>
<td>O26, O103, O145</td>
<td>Ireland</td>
<td>0.5%, 5.5%, 0.7%</td>
<td>Thomas et al., 2012</td>
</tr>
<tr>
<td>Feces (399)</td>
<td>O26, O103, O111 O145</td>
<td>Belgium</td>
<td>6%, 6%, 6%, 6%</td>
<td>Joris et al., 2011 (47)</td>
</tr>
<tr>
<td>Carcass (474)</td>
<td>O157</td>
<td>Denmark</td>
<td>3.4%</td>
<td>Breum et al., 2010 (155)</td>
</tr>
<tr>
<td>Carcass (1,622)</td>
<td>O157</td>
<td>Argentina</td>
<td>2.6%</td>
<td>Masana et al 2010 (156)</td>
</tr>
<tr>
<td>Feces (1,622)</td>
<td>O157</td>
<td>Argentina</td>
<td>4.1%</td>
<td>Masana et al, 2010</td>
</tr>
<tr>
<td>Ear (446)</td>
<td>O157</td>
<td>Sweden</td>
<td>12%</td>
<td>Boqvist et al., 2009 (157)</td>
</tr>
<tr>
<td>Feces (1,758)</td>
<td>O157</td>
<td>Sweden</td>
<td>3.4%</td>
<td>Boqvist et al., 2009</td>
</tr>
<tr>
<td>Sheep</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fleece (500)</td>
<td>O157</td>
<td>Ireland</td>
<td>0.8%</td>
<td>Thomas et al., 2013 (61)</td>
</tr>
<tr>
<td>Fleece (500)</td>
<td>O26, O103, O145</td>
<td>Ireland</td>
<td>1.0%, 16.8%, 0.2%</td>
<td>Thomas et al., 2013</td>
</tr>
<tr>
<td>Carcass (500)</td>
<td>O157</td>
<td>Ireland</td>
<td>0.6%</td>
<td>Thomas et al., 2013</td>
</tr>
<tr>
<td>Carcass (500)</td>
<td>O26, O103, O145</td>
<td>Ireland</td>
<td>0.4%, 13.6%, 0</td>
<td>Thomas et al., 2013</td>
</tr>
<tr>
<td>Feces (17,550)</td>
<td>O103</td>
<td>Sweden</td>
<td>0.7%</td>
<td>Sekse et al., 2013 (58)</td>
</tr>
<tr>
<td>Feces (492)</td>
<td>O157</td>
<td>Sweden</td>
<td>1.8%</td>
<td>Soderlund et al., 2012 (51)</td>
</tr>
<tr>
<td>Ear (105)</td>
<td>O157</td>
<td>Sweden</td>
<td>1.9%</td>
<td>Soderlund et al., 2012</td>
</tr>
<tr>
<td>Feces (1,082)</td>
<td>O157</td>
<td>Scotland</td>
<td>3.4%</td>
<td>Evans et al., 2011 (56)</td>
</tr>
<tr>
<td>Feces (1,082)</td>
<td>O26, O103, O145</td>
<td>Scotland</td>
<td>3.4%, 5.2%, 2.3%</td>
<td>Evans et al., 2011</td>
</tr>
<tr>
<td>Feces (491)</td>
<td>O26</td>
<td>Norway</td>
<td>17.9%</td>
<td>Sekse et al., 2011 (52)</td>
</tr>
<tr>
<td>Feces (533)</td>
<td>O157</td>
<td>Italy</td>
<td>7.1%</td>
<td>Franco et al., 2009 (54)</td>
</tr>
<tr>
<td>Feces (400)</td>
<td>O157</td>
<td>Ireland</td>
<td>5.75%</td>
<td>Lenahan et al., 2007 (60)</td>
</tr>
<tr>
<td>Carcass (400)</td>
<td>O157</td>
<td>Ireland</td>
<td>1.5%</td>
<td>Lenahan et al., 2007</td>
</tr>
</tbody>
</table>

1Individual fecal swabs were collected from cattle approximately 60 days after their arrival in the feedlot and were pooled for evaluation (153).
2The investigation of fecal samples from 585 sheep flocks resulted in 1,222 E. coli O103 isolates that were analyzed for the presence of eae and stx genes; the study documented a low prevalence (0.7%) of isolates potentially pathogenic to humans (58).
3This number represents the number of flocks that were tested in the study using fecal samples to determine the prevalence; in total, 491 flocks were tested and E. coli O26 was detected in 17.9% of the flocks (53).
phenomenon of “super-shedding” animals (those shedding $>10^4$ CFU/g feces) is thought to be a significant contributor in the dissemination of O157 VTEC within and between herds and within abattoirs (35–37). However, quantitative data are few relative to prevalence data as the routine detection methods generally employed in surveys are designed to yield data only on presence of the pathogen and not on the numbers present. As a result, data on concentrations of VTEC and on occurrence of super-shedders are limited. Thomas et al. (38) examined feces and hide for concentrations of six VTEC serogroups and showed that the vast majority of samples had counts below the limit of detection of the count method and samples with detectable counts ranged from 60 to 100 CFU/cm² on hide and 100 to 1300 CFU g⁻¹ in feces. An abattoir study in the United Kingdom found that 70% of E. coli O157-positive animals shed <100 CFU g⁻¹ of feces, but in some individuals concentrations could be as high as $10^6$ CFU g⁻¹ of feces (39). These authors also showed that 9% of the animals shedding E. coli O157:H7 at slaughter produced over 96% of the total O157:H7 fecal load for the group. It has also been hypothesized that high-level carriage of these microorganisms is a consequence of intestinal colonization whereas low levels within individual animals may be a result of environmental exposure with no significant colonization (32, 40). Horizontal transmission between animals may be facilitated by contaminated water and feed, with water troughs potentially playing a role in the ecology of VTEC on the farm (41–46).

Recent studies have examined the human virulence potential of different bovine VTEC isolates, and an interesting picture is seen for O157 versus non-O157 VTEC serogroups. Whereas >90% of E. coli O157 isolates recovered from the beef chain in Ireland possessed vt and eae in combination, <10% of non-O157 VTEC serogroups fell into this category (38). In a Belgian study on O26, O103, O145, and O111 in cattle feces, about 6% of samples were positive and about 50% of isolates had key human virulence genes (47, 48). A further study showed that for E. coli O157 genotypes associated with human illness a minor subpopulation was in the bovine reservoir. (47, 48) This shows that while VTEC isolates in beef play a role in human illness, a risk assessment of their virulence potential is essential.

**Carriage of VTEC in Sheep and Goats**

The presence of naturally occurring VTEC has been widely reported in sheep, and studies indicate that sheep feces and sheep meat are important reservoirs of human pathogenic VTEC (49–55). Seasonal prevalence of VTEC in sheep has been reported (54, 56), with incidences peaking in summer months in a similar trend to that in cattle and human infections (45). Much of the data on VTEC colonization of small ruminants relate to E. coli O157. When colonized, small ruminants generally show no clinical symptoms of illness and reinfection occurs frequently, although in young unweaned lambs or kids, scouring or diarrhea may occur. Some animals, particularly those that are persistently colonized, can excrete exceptionally high numbers of E. coli O157 (>10,000 CFU/g) in their feces (54, 57). There is some evidence of higher shedding of O157 in adult sheep and hoggets than in lambs (56).

E. coli O103 is one of the most common non-O157 VTEC serotypes isolated from human cases in Europe, and a severe human outbreak was reported in Norway in 2006 that was caused by E. coli O103:H25 (38). This was due to fermented sausage mainly consisting of mutton, which was shown to be the food source of the outbreak (59). However, only eae vt E. coli O103:H25 was ever isolated from food and sheep, but it was proposed that the infection was caused by VTEC and that recovered isolates probably had lost their vt genes during laboratory cultivation. In studies carried out in Ireland, the profile of E. coli O157:H7 recovered from sheep showed that 29/33 (87%) of isolates contained vt2, eae, and blyA genes, whereas only five isolates (15%) contained the vtX1 gene (60, 61). Thomas et al. showed recovery of pathogenic O26, O103, and O145 (with eae and vt) at level <1% on sheep fleece and carcasses (61). For O26, isolates with vt and eae represented about 50% of all isolates of the serogroups recovered, while for O103 it was only about 0.01% of all isolates. As for bovine isolates, these data highlight the need to risk assess any recovered VTEC regardless of serogroup for key virulence genes (61).

**Carriage of VTEC in Pigs**

Although pigs are also known carriers of O157 VTEC, prevalence rates are relatively low, ranging from 0.4 to 2.1% (62–65). In pigs, VTEC can cause edema disease and may also be involved in postweaning diarrhea syndromes. In general, it is VTEC serogroups O138, O139, and O141 that are implicated in illness in pigs, and these have not been widely linked to human illness. The presence of E. coli O157 in pigs has been reported in the United States (62), Norway, and the Netherlands (66, 67). In a study carried out in Ireland, the prevalence of E. coli O157:H7 in pigs was found to be very low,

**Verocytotoxigenic Escherichia coli and Veterinary Public Health**
and only four isolates were recovered from 1,710 pigs examined. However, three of these four contained the genes \textit{vtx}2, \textit{eae}, and \textit{hly}A, indicating their potential to cause illness in humans (68). While pork meat and products have generally not been implicated in human illness, in California in 2006, feral swine were implicated in contamination of agricultural fields and surface waterways with \textit{E. coli} O157:H7, which caused a large outbreak linked to bagged spinach. However, \textit{E. coli} O157 isolates were also recovered from cattle on a nearby ranch that had a similar subtype to the swine isolates as determined by using multilocus variable tandem repeat analysis and pulsed-field gel electrophoresis (PFGE), suggesting that not only had swine-to-swine transmission taken place but, in addition, that interspecies transmission between cattle and swine had occurred, facilitated through a common source of exposure such as water or soil (69). More data on the human virulence assessment of non-O157 serogroups isolated from pigs would provide important information on the likely future importance of their role as a vector of human VTEC infection.

**Survival of VTEC in Animal Waste**

Animal waste in the environment can arise from feces directly excreted into the environment by grazing or wild animals and from animal waste collected and stored as slurry or manure. Manures are feces that may have undergone some period of storage, and slurries are mixtures that include manure, urine, and leftover feed that are held in a tank or pit, generally under anaerobic conditions. Such waste is a valuable source of soil nutrients and is generally spread back to the land as fertilizer. If such waste is contaminated with VTEC, it poses a reservoir of contamination for animals, water supplies, crops, and fresh produce.

In experiments on the survival of \textit{E. coli} O157:H7 in feces spread outdoors on grass under ambient weather conditions, the pathogen could be recovered directly from feces on the grass for 30 days, and when feces were no longer visible on the grass, enrichment techniques showed the organism was recoverable from the underlying soil for a further 49 days (71). A similar study in the United Kingdom (72) reported the survival of \textit{E. coli} O157 in cattle feces for >50 days but reported much shorter survival times in cattle slurry in which it fell to undetectable levels within 10 days. However, McGee et al. reported that \textit{E. coli} O157:H7 was recoverable from bovine slurry for up to 3 months (73). Semenov et al. showed the influence of aerobic and anaerobic conditions on the survival of \textit{E. coli} O157:H7 in cattle manure and slurry (74). The data showed that \textit{E. coli} O157:H7 survived significantly longer under anaerobic than under aerobic conditions, with survival ranging from approximately 2 weeks for aerobic manure and slurry to more than 6 months for anaerobic manure at 16°C. The importance of changes in microbial community and chemical composition of manure and slurry was highlighted as affecting the survival of \textit{E. coli} O157:H7 in different oxygen conditions.

In further field experiments to determine the survival times of pathogens in livestock manures during storage and following land application, VTEC survived for less than 1 month in solid manure heaps where temperatures greater than 55°C were obtained. Following spreading of manure to land, \textit{E. coli} O157 generally survived in the soil for up to 1 month after application to both sandy arable and clay loam grassland soils (75).

When left on the ground surface, pathogens such as VTEC are exposed to the full force of fluctuating environmental conditions that has been shown to increase the inactivation rate as compared to the immediate incorporation into the soil (75, 76). It has been found that \textit{E. coli} O157 can survive on surface vegetation for
VTEC in Water

Surface water and unprotected groundwater may become contaminated with VTEC from livestock effluent or fecal contamination from humans, livestock, wild animals, and domestic pets. Water for both drinking and recreational swimming has been implicated in a number of VTEC outbreaks (86). The susceptibility of drinking water from private water supplies in rural areas to VTEC contamination has been highlighted, with a private water supply being considered as one that is not provided by a water company and includes wells, boreholes, springs, streams, rivers, lakes, or ponds. Such private water supplies are common in both Ireland and Scotland, where rates of human VTEC illness are high, and in both countries private water can be considered a potentially important risk factor for VTEC human infection (87–89). A large E. coli O157 outbreak involving a private group water scheme was reported in a rural area of Ireland, involving 18 cases of infection, including 2 HUS cases (46). A study by Tanaro et al. showed that on a beef farm in Argentina, non-O157 isolates with identical PFGE profiles were recovered from cattle rectal swabs, and water streams on the farm highlighted the circulation of VTEC strains among the animals, the environment, and the water course (90). Other studies have shown that the vulnerability of groundwater supplies is influenced by soil types, with groundwater being most at risk where the subsoils are absent or thin and in areas of karstic limestone, where surface streams sink underground at swallow holes (91, 92).

Studies have shown that E. coli O157:H7 survived for 13 weeks in lake water held at 15°C (93), while other studies have reported survival in farm water for up to
14 days at temperatures of <15°C (94), 70 days at 5°C, and 40 days at 21°C (95). In addition to temperature, the indigenous microflora (93), scarcity of nutrients (96), and protozoan predations also influence survival. Recent passage of VTEC through the bovine gastrointestinal tract may enhance survival of O157 in water (97).

Contaminated water troughs and livestock drinking water supplies have a role in the transmission of VTEC among animals (33), and large numbers of animals can be infected over a short period (42). VTEC can survive in water trough sediments for at least 4 months and appears to multiply there, especially in warm weather. On many farms, troughs are seldom cleaned so that thick sediments accumulate and remain a long-term potential source for continual recycling of VTEC among a herd. Farmers should clean water troughs frequently to prevent the accumulation of sediments (42). Water trough design and location are also important factors in reducing the possibility of direct fecal contamination. Water troughs should be positioned away from feed troughs/feed passageways, as contamination of water with feed can provide a nutrient-rich substrate for bacterial growth and survival at the bottom of the trough (42). Water troughs should not be located in shaded areas (42), as direct sunlight has a bactericidal effect.

VTEC IN FOODS OF ANIMAL ORIGIN

Food of animal origin such as milk and dairy products may be contaminated with VTEC during milking, and the meat carcass may be contaminated during slaughter and dressing operations.

VTEC in Milk

Although the public health risks associated with consumption of raw milk and raw milk products have been well documented, such products continue to be consumed and promoted by pro-raw milk advocates because of perceived organoleptic and health benefits over pasteurized products (98, 99). However, a number of serious outbreaks of E. coli O157 have been associated with the consumption of raw milk (100, 101), raw milk cheese (102), and unpasteurized Gouda cheese (103, 104). Other outbreaks were linked to butter made from raw milk and to commercial ice creams, where cross-contamination was identified as a possible source (105). Allerberger et al. reported two cases of HUS due to E. coli O26 linked to the consumption of raw cow’s milk (106). However, pasteurized products have also been implicated in outbreaks, and E. coli O145 and O26 caused VTEC infection with five HUS cases among consumers of ice cream produced and sold in September 2007 at a farm in Belgium. The ice cream was made from pasteurized milk and most likely was contaminated by one of the food handlers (107). An outbreak of E. coli O111 associated with a correctional facility dairy in Colorado showed inmates employed at the dairy might have acquired VTEC O111 infection on the job or transported contaminated clothing or other items into the main correctional facility and kitchen, thereby exposing other inmates (108).

Contamination of raw milk can occur either during milking or after milking from on-farm environmental contamination. A study by Murphy et al. examined milk from 97 Irish dairy cattle farms and isolated E. coli O157:H7 from 12% of samples on these farms (109). A subsequent study by Murphy et al. examined Irish raw milk specifically destined to be used in raw milk cheese and detected VTEC in raw milk from sheep and goats in addition to cows (110). Data collated from European Union member states in 2011 (111) showed 5.3% (3/57) of raw milk samples in Germany contained VTEC and 2.6% (1/39) raw milk samples in Belgium contained E. coli O157. Among five reporting member states, 1.8% (36/2,045) of all dairy samples were positive for VTEC and 0.1% for E. coli O157. Between 2008 and 2011, an Italian study that monitored for the prevalence of E. coli O157:H7 in raw milk (60,907 samples) sold by self-service vending machines (n = 1,239) in seven Italian regions found just 24/60,907 (0.04%) of samples were positive for E. coli O157:H7.

VTEC may reportedly behave differently in raw and pasteurized milk and in derived products. Growth of VTEC may be slower in raw milk and products because of the presence of lactoperoxidase enzymes, natural inhibitors for many pathogenic bacteria in raw milk, and may also be due to the higher numbers of competitive microflora in such products (112). The behavior and the potential for survival/growth of VTEC in dairy products such as cheese depend on factors such as the moisture content, pH, and competitive microflora. Thus the additional hurdles imposed during the hard cheese manufacturing process, including low water activity and pH that develop during the curing process, and the changes in the competing microflora, reduce the survival and growth potential of the pathogen. Peng et al. showed survival of non-O157 following the production and ripening of semi-hard raw milk cheese (113). Miszczyn et al. examined the growth and survival of four VTEC serotypes (O157:H7, O26:H11, O103:H2, and O145:H28) in raw-milk cheeses manufactured and
ripened according to five technological schemes: blue-
type cheese, uncooked pressed cheese with long ripening
and short ripening steps, cooked cheese, and lactic
cheese (114). VTEC grew 2 to 3 log10 CFU g−1 in the
blue-type cheese and the two uncooked pressed cheeses
during the first 24 h of cheese making, but levels then
progressively decreased in cheeses that were ripened for
more than 6 months. In the cooked and lactic cheeses,
VTEC did not grow but was detectable at the end of
ripening and storage. Interestingly, a serotype effect
was observed in all cheeses, with O157:H7 growing
slower and less persistently than the other serotypes,
indicating that the risk posed by non-O157 serotypes
may be higher and the design of safety measures should
take this into account.

Several studies reported the ability of E. coli O157:H7
to survive and to grow during storage of fermented dairy
products. E. coli O157:H7 inoculated into commercial
products could be recovered for up to 12 days from
yogurt, 28 days from sour cream, and 32 to 35 days from
buttermilk (115, 116).

**VTEC and Meat**

Since the first confirmed case in 1982, beef-associated
VTEC O157 outbreaks were widely reported (3, 117–119).
Between 2007 and 2009, of the 57 verified food-
borne outbreaks of VTEC in the European Union, 8 were
linked to the consumption of bovine meat or bovine
meat products. During the years 2010 and 2011, of the
16 outbreaks reported with strong evidence, 2 were
linked to the consumption of bovine meat or bovine
meat products (6).

The hide and fleece of animals presented for slaughter
are recognized as important sources of VTEC contami-
nation for the carcass. Reported prevalence of E. coli
O157 on hide varies from 4.7% (120), 17.6% (38), to
75.7% for feedlot cattle (121). On sheep fleece the
reported prevalence of E. coli O157 was 5.75 (60) and
<1% (61). Studies looking at an extended range of
VTEC serogroups (O26, O111, O145, O103) recovered
<1% on bovine hide and on sheep fleece recovered O26
at 1%, with neither O103, O145, nor O111 recovered
(38, 61).

Transmission of E. coli O157:H7 and other VTEC
serotypes can occur rapidly in groups of animals, with
contamination of the bovine pens and hides occurring in
less than 24 h (122). Thus conditions in transport from
farm to factory and in lairage have a significant impact
on the level of VTEC contamination on hide or fleece
of animals presented for slaughter. The risk of intro-
ducing VTEC to a slaughter batch is increased by mixing
animals from different farms (123, 124). The length of
time animals are in transit to the abattoir and held in
lairage also increases the risk of having VTEC-positive
hide samples at slaughter, compared with cattle trans-
ported a shorter distance (125, 126). E. coli O157 has
been shown to survive on the hides of live cattle for
approximately 9 days. Efforts to reduce the level of
hide or fleece soiling are thus warranted for control of
VTEC. Farmers can employ suitable animal husbandry
facilities and practices, such as bedding quality, stocking
density, and feeding regimen, that give clean animals for
slaughter although a clean animal does not guarantee
absence of VTEC. Livestock transporters can also play
an important role in the cleanliness and dryness of ani-
malson arrival at the abattoir by ensuring that trucks
are thoroughly washed and disinfected between loads and by not overloading the animals.

For the abattoir, knowledge from the farm of the
VTEC prevalence in the slaughter batch could allow
risk management of animals coming in for slaughter,
including logistic slaughter, but currently there is insuf-
ficient information on VTEC at the farm level to im-
plement this approach (6, 127).

The risk of fecal contamination on the carcass at
slaughter can be reduced by specific procedures, in-
cluding “rodding” (a technique used to separate the
esophagus from the trachea and diaphragm). Bagging
and tying of the bung can also help prevent contami-
nation of the carcass. Removal of hides should be carried
out in a manner that avoids contact between the hide
and the carcass. This can be achieved by a number of
measures, including the use of hide pulling equipment
and using clean equipment (immersion of knives in
water at 82°C) for the dehiding operation.

The reported prevalence of E. coli O157 on prechill
carcasses (postevisceration) is low (≤3%) (22). There is
a low prevalence (<0.5%) of other clinically significant
VTEC serogroups (O145, O111, O103, and O26) on
prechill beef and sheep carcasses (38, 61). The similarity
of PFGE profiles for VTEC isolates in a study by Thomas
et al. indicated the origin of VTEC on a carcass could be
its own hide or the hides or fleece of other animals being
slaughtered on the same day (38, 61). VTEC that may
also be present in the air of the slaughter house, par-
icularly at the hide removal area, may be an under-
estimated source of carcass contamination (128). The
use of carcass antimicrobial interventions is common-
place in the U.S. beef industry (129) and include the use
of organic acids, acidified sodium chlorite, trisodium
phosphate, activated lactoferrin, chlorine, and chlorine
dioxide and hot water. Studies by Kalchayanand et al.
indicated that the reductions in non-O157 VTEC by the commonly used antimicrobial interventions were at least as great as for O157 (130). Such antimicrobial interventions for beef carcasses are not currently in use in the European Union beef industry sector. This is mainly due to customer preferences and associated costs.

The survival and concentrations of E. coli O157:H7 and other VTEC serogroups on fresh meat during distribution can be affected by the storage temperatures (131, 132), the packaging environment (133), and the competitive microflora (134). The processing of beef cuts into ground beef can lead to transfer of pathogen from the surface of beef into the center of the product, and beef-processing equipment and knives or needles used to cut into or inject whole muscle (in tenderizing beef) can play a role in this spread of contamination (135–137). Studies on thermal inactivation of different single serotypes of VTEC O26:H11, O45:H2, O103: H2, O104:H4, O111:H1, O121:H19, O145:NM, and O157:H7 in wafers of ground beef showed that cooking times and temperatures effective for inactivating a serotype O157:H7 strain of E. coli in ground beef were equally effective against the seven non-O157:H7 strains investigated (135).

VTEC can reportedly survive in ready-to-eat, dry, and semidry fermented meats, posing a particular risk in these commodities (138). This has led to a recommendation that processing protocols achieve a log_{10} 5.0 CFU g^{-1} reduction in numbers of E. coli O157:H7. Such reductions may be achieved by additional thermal processing, and a number of published studies outline heating steps that can be introduced into the manufacturing process after fermentation to achieve the required decline in pathogen numbers (139). In the absence of a thermal processing step, an extended fermentation or maturation period may prove effective in limiting pathogen numbers.

CONCLUSION

It is clear that there are multiple routes for transmission of human pathogenic VTEC along the farm-to-fork chain. Emerging challenges are new vehicles of infection and a changing profile of the VTEC serogroups that are causing human illness. A lack of clarity on what constitutes a human pathogenic VTEC poses additional challenges in assessing and managing public health risk. Whole genome sequencing approaches are now helping identify the genetic differences between human pathogenic and nonpathogenic VTEC, but more research is needed into the interactions between virulence factors and the human or animal host. Much of the knowledge to date has been obtained from in vitro observations, which do not necessarily reflect what is happening in vivo within a specific host. It is also likely that the microbiota plays a crucial part in disease dynamics and in host-pathogen and host-commensal and commensal-pathogen interactions. For example, signals from the commensal flora may affect expression of virulence determinants in pathogens (140). Therefore, the challenges that lie ahead are to improve the understanding of the ecology of the pathogen at sites of colonization and in key contamination in the agri-food environment that will underpin strategies for the prevention of transmission to target this diverse group of pathogens.

In addition, among members of a serotype there may be significant strain differences that warrant further research and that need to be considered for effective management practices. Data are now building on the phenotypic characteristics and behavior of non-O157 VTEC in the food chain that indicate that they behave in a similar way to O157 and that current general interventions implemented at pre- or postharvest stages of the food chain will yield similar reductions regardless of serogroup (130, 135, 141). However, there are some recent studies that indicate that this may not always be the case, i.e., in semi-hard raw-milk cheese, non-O157 VTEC persisted longer than O157 (114). There is an urgent need for robust studies with more serogroups and more strains to validate survival kinetics of diverse VTEC strains and to assess the impact of food control measures to ensure that differences are true serogroup differences and not related to an interstrain impact. There is a need for better epidemiological linkage of strains from animal, farm environment, and foods and humans and for source attribution of VTEC, in particular, for emerging serogroups.

This is challenging, but with so many potential vehicles and routes of infection, some risk ranking at a country or regional level of the pathways and products, based on the relevant regional epidemiological and practices, would be useful in effectively directing and focusing veterinary public health risk management approaches and resources.

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REFERENCES

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