



Review

A review of factors that affect transmission and survival of verocytotoxigenic *Escherichia coli* in the European farm to fork beef chain



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ABSTRACT

Verocytotoxigenic *Escherichia coli* (VTEC) are a significant foodborne public health hazard in Europe, where most human infections are associated with six serogroups (O157, O26, O103, O145, O111 and O104). With the exception of O104, these serogroups are associated with bovine animals and beef products. This paper reviews our current knowledge of VTEC in the beef chain focusing on transmission and the factors which impact on survival from the farm through transport, lairage, slaughter, dressing, processing and distribution, in the context of the European beef industry. It provides new information on beef farm and animal hide prevalence, distribution and virulence factors as well as pre-chilled carcass and ground beef prevalence, generated by the recently completed EU Framework research project, *ProSafeBeef*. In the concluding section, emerging issues and data gaps are addressed with a view to increasing our understanding of this pathogen and developing new thinking on detection and control.

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1. Introduction

Verocytotoxigenic *Escherichia coli* (VTEC), also referred to as Shiga-toxin producing *E. coli* (STEC), are a group of *E. coli* that carry verocytotoxin (*vtx*) genes encoded on lamboid lysogenic bacteriophage. They are important pathogens which cause mild to severe illness in

humans. Ruminant animals, including cattle, are an important reservoir of VTEC which is shed in their faeces. The pathogen is then transmitted to humans either by direct contact with VTEC contaminated faecal material, or indirectly via consumption of faecally contaminated water or food. Meat may be contaminated during slaughter and dressing operations. Foods of bovine origin, especially undercooked beef products, have been linked to approximately 75% of O157 VTEC outbreaks in the US (Callaway, Carr, Edrington, Anderson, & Nisbet, 2009). *E. coli* O157 was the first VTEC associated with human illness (Riley et al., 1983)

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and remains the most commonly isolated human pathogenic VTEC serogroup from the beef chain. Therefore, research has tended to predominantly focus on this serogroup.

Whilst some cases of VTEC infection present with uncomplicated non bloody diarrhoea, others will show severe symptoms including haemorrhagic colitis (HC) with bloody diarrhoea. In approximately 20% of cases, life-threatening complications then occur, of which haemolytic uraemic syndrome (HUS) is the most common. Around 3–5% of cases are fatal. In the USA and Canada there has been a decreasing trend in O157 VTEC infections in recent years, with the former reporting a significant decrease from 2006–2008 to 2011 (CDC, 2012) and the latter reporting a decrease in incidence rate from 3.00 to 1.39/100,000 from 2006 to 2011 (NESP, 2012). In contrast in Europe there has been an increasing trend in the reporting of human VTEC cases in the same period (ECDC, 2013). This does not include a large O104 VTEC outbreak (3842 cases) which occurred predominantly in Germany with 855 HUS cases and 53 deaths (Beutin & Martin, 2012). It should be noted this outbreak was considered unusual and not beef associated. In recent years confirmed cases of non-O157 VTEC infection in the human population have equalled or surpassed those caused by VTEC O157 in both Europe and the USA (Bosilevac & Koohmaraie, 2011; EFSA, 2007). It is difficult to track the source of many cases due to their sporadic nature but ruminants are still considered the main reservoir. A recent EFSA Scientific Opinion identified VTEC as high priority biological hazards for meat inspection of bovine animals (EFSA, 2013a).

The VTEC group of bacteria comprises a large number of serogroups and cattle can carry a very diverse range. Such diversity is based on the spread of *vtx* genes to various serogroups by bacteriophage mediated horizontal gene transfer (Herold, Karch, & Schmidt, 2004). The serogroups most often associated with severe human VTEC illness are O157, O26, O103, O145 and O111 in Europe (EFSA, 2013b) and O157, O26, O45, O103, O111, O121 and O145 in the USA (USDA-FSIS, 2012). All of the main serogroups have been reported in ruminant animals, especially bovines, and have been epidemiologically linked to large outbreaks and serious illness and/or fatalities in humans. A selection of beef associated VTEC outbreaks are shown in Table 1. Analysis of foodborne disease outbreaks in the USA in 2009 and 2010 indicated that the pathogen–commodity pair responsible for the most deaths was O157 VTEC and beef, with three deaths (CDC, 2013). Increasingly, outbreaks have been associated with non O157 VTEC serogroups. However, as only a minority of VTEC serogroups recovered from beef chain are likely to be pathogenic to humans, it highlights the importance of assessing any recovered isolates for virulence related genes. A recent EFSA Scientific Opinion (2013b) proposes that any isolate 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 diarrhoea and HUS. For any other VTEC serogroups with the same

combination of virulence genes, the potential risk is regarded as high for diarrhoea, but currently unknown for HUS. In the absence of these genes, currently available data does not allow any inference regarding potential risks.

This review focuses on the transmission and survival of VTEC in the beef chain in Europe and presents some recent research conducted as part of an EU Framework research project *ProSafeBeef*. The objective of this large multi-partner project was to improve beef safety and quality through research and innovation, with a particular emphasis on VTEC prevalence and virulence in the beef chain.

2. Occurrence in cattle

Cattle, and to a lesser extent other ruminant animals, are the primary reservoir of VTEC (Caprioli, Morabito, Brugreb, & Oswald, 2005). In the USA, most bovine animals have been exposed to O157 VTEC by the time of weaning (Laegreid, Elder, & Keen, 1999) and the reported prevalence in cattle ranges from 10% to 28% (Karmali, Gannon, & Sargeant, 2010). In the EU, individual animal, herd and slaughter batch VTEC prevalence range from 2.1% to 13.5%, 6.1% to 12.6% and 13% to 20.2%, respectively and from 0.2% to 2.3%, 1.5% to 13.7% and 5.5% to 20.2%, respectively for *E. coli* O157 (EFSA, 2013b). Cattle are also the primary source of non-O157 VTEC (Bosilevac & Koohmaraie, 2011). Monaghan et al. (2011, 2012) recently reported that 40% of bovine faecal samples taken from 20 Irish beef farms were *vtx* gene PCR positive. In Italy, 3.6% of slaughter cattle were non-O157 positive (Bonardi et al., 2004), whilst in Germany 1.2%, 0.75% and 0.5% of cattle carried O26, O111 and O145, respectively (Wieler et al., 1996). Joris, Pierard, and De Zutter (2011) studied the prevalence of *E. coli* O26, O103, O111 and O145 in Belgian cattle and reported that 6% of faecal samples were positive, with O26 and O103 being more abundant in slaughter cattle than O111 or O145. Scottish cattle have O26, O103, O145 and O111 carriage rates of 4.6%, 2.7%, 0.7% and 0%, respectively (Pearce et al., 2006).

The prevalence, distribution and virulence factor profiles of VTEC serotypes on twelve beef farms within the same geographical area in the north-east of Ireland were investigated as part of the *ProSafeBeef* project. Of the 650 samples analysed, 13.7% (89/650) were *vtx* PCR positive. Although O157 was also the most prevalent serotype, 32 other serotypes were also obtained including; O–:H–, O–:H10, O–:H11, O–:H12, O–:H14, O–:H16, O–:H18, O–:H21, O–:H46, O–:H48, O2:H+, O2:H25, O2:H27, O2:H32, O3:H12, O26:H11, O33:H11, O69:H–, O76:H34, O88:H8, O113:H4, O113:H36, O118:H16, O136:H12, O150:H8, O153:H+, O153:H40/44, O157:H16, O171:H2, OR:H18, OX18:H38 and OX18:H+. The O–H–, O26:H11, O76:H34, O157:H7, O157:H16 and OX18:H+ serotypes also carried the *eae* and *hlyA* genes. Other virulence genes; *tir*, *saa*, *espA*, *espB*, *iha*, *lpfA*_{O157/O1-141}, *lpfA*_{O157/O1-154}, *toxB*, *etpD*, and *katP* were present in 45%, 17%, 35%, 40%,

Table 1
Selected international VTEC outbreaks (2007–2013) associated with the consumption of beef or beef products.

Country	Serogroup	Year	Number of illnesses (reported deaths)	Vehicle of transmission	Reference
Denmark	O157	2012	9	Ground beef	Soborg, Muller, Lassen, Ethelberg, and Molbak (2012)
Netherlands	O157	2011	3	Filet American	EFSA (2013b)
UK	O157	2011	4	Beef curry	EFSA (2013b)
Japan	O111	2011	181 (5)	Yukhoe (raw beef dish)	National Institute of Infectious Diseases, Japan (2012)
USA	O26	2010	3	Ground beef	USDA-FSIS (2012)
USA	O157	2010	21	Blade tenderised steak	CDC (2010a)
France	O123	2009	2	Ground beef	King et al. (2010)
USA	O157	2009	26 (2)	Ground beef	CDC (2009a)
USA	O157	2009	23	Ground beef	CDC (2009b)
Netherlands	O157	2008–2009	20	Steak tartare	Greenland et al. (2009)
Belgium	Not reported	2008	6	Raw minced beef	EFSA (2013b)
USA	O157	2008	49	Ground beef	CDC (2008)
USA	O157	2008	99	Beef cuts	CDC (2010b)
Denmark	O26	2007	18	Beef sausage	Ethelberg et al. (2007)
USA	O157	2007	40	Ground beef patties	CDC (2007)

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