Risk Profile on Non-O157 Verotoxin-Producing *Escherichia Coli* in Produce, Beef, Milk and Dairy Products in Canada

Regular Paper

Angela Catford1,*, Valentin Kouamé1, Amalia Martinez-Perez1, Alexander Gill1, Enrico Buenaventura1, Hélène Couture1 and Jeffrey M Farber1

1 Bureau of Microbial Hazards, Food Directorate, Health Products and Food Branch, Health Canada, Sir FG Banting Research Centre, Ottawa, ON, Canada

* Corresponding author E-mail: angela.catford@hc-sc.gc.ca

Received 20 May 2014; Accepted 10 Aug 2014

DOI: 10.5772/59208

© 2014 The Author(s). Licensee InTech. This is an open access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/3.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Abstract This risk profile on non-O157 verotoxin-producing *Escherichia coli* serotypes (non-O157 VTEC) in produce, beef, milk and dairy products in Canada, outlines associated potential public health threats, while identifying areas where additional data are required to inform microbiological risk assessment and risk management action. The following outputs of this risk profile may contribute to risk analysis relating to non-O157 VTEC in Canada:

- **Beef products, milk and dairy products, and produce** are all food commodities associated with non-O157 illness outbreaks. Some non-O157 VTEC may cause foodborne illnesses similar to *E. coli* O157:H7, however in general, non-O157 VTEC cause less severe illness or lead to asymptomatic infections. In the absence of epidemiological evidence (i.e., the inability to show that the detected bacteria has previously caused illness in humans), the ability to establish the health concern of isolates, is limited.

- **Non-O157 VTEC serogroups most commonly implicated in human infections differ between countries. In Canada, the top six non-O157 VTEC serogroups associated with serious illness are: O26, O103, O111, O117, O121, and O145.**

- The dose-response(s) for non-O157 VTEC in beef products, milk and dairy, and produce as groups is currently not possible to determine.

- VTEC are defined by the production of verotoxins. Other virulence factors are associated with clinical isolates of non-O157 VTEC and may indicate an increased threat to human health, but a common virulence profile for pathogenic VTEC has not been identified.

- The government of Canada commissioned the Federal VTEC Working Group to develop detection and isolation methods for non-O157 VTEC as an initial regulatory response to support the reporting of non-O157 VTEC in foods. A Canadian VTEC method collaboratively developed by Health Canada and the Canadian Food Inspection Agency is being used to detect the presence of non-O157 VTEC in food.
Various food processing interventions in the commodities investigated in this risk profile have been shown to equally control both *E. coli* O157:H7 and non-O157 VTEC.

**Keywords** Non-O157 VTEC, Risk Profile, Canadian Data, Beef, Dairy, Produce

1. Introduction

Verotoxin-producing *Escherichia coli* (VTEC) were first identified in the late 1970s [1]. VTEC are distinguished from other enteric pathotypes of *E. coli* by the expression of one or more verotoxins. Verotoxins are closely related to the shiga toxin of *Shigella dysenteriae* and the similarity between these toxins has given rise to a competing terminology of shiga-like toxins and shiga toxin producing *E. coli* (STEC). The terms shiga-like toxin and STEC are interchangeable with the terms verotoxin and VTEC.

The clinical significance of VTEC was established in 1983, with the report of VTEC serotype O157:H7 as a cause of hemorrhagic colitis (HC), and hemolytic uremic syndrome (HUS) [2]. Although *E. coli* O157:H7 has been implicated in many outbreaks, there is increasing recognition that VTEC of other serotypes (non-O157 VTEC) are also a significant cause of human disease [3]. For example, in 2011, Europe experienced an outbreak of VTEC foodborne illness which resulted in a total of 4075 confirmed cases and 50 deaths linked to the consumption of fenugreek sprouts contaminated with *E. coli* O104:H4 [4]. This outbreak is the largest in reported history due to non-O157 VTEC contamination of food.

More than 200 different serotypes of non-O157 VTEC have been associated with human illness [5]. Epidemiological reports indicate that in Canada and abroad, the most common serotypes of VTEC implicated in human infections differ between geographic regions. Non-O157 VTEC are now recognized to cause greater than 50% of VTEC illnesses in the U.S. [6]. Similarly, Canadian surveys of diarrheal illness indicate that approximately 50% to 60% of VTEC illnesses are caused by non-O157 VTEC [7-9].

Domestic and wild animals, in particular ruminants, are known reservoirs of VTEC. Although non-O157 VTEC are not newly emerging pathogens [1, 10] and infection with non-O157 VTEC continues to be associated with sporadic cases and outbreaks of enteric illness, due to the relative expense and complexity of testing, very few clinical laboratories routinely test for these pathogens [11].

Overall, there is a significant amount of information which needs to be collected regarding the role of non-O157 VTEC in foods. The majority of what is known is from outbreak data and the current path forward for research is focused on development of methodologies to appropriately understand the biology of these bacteria, and distinguish pathogenic from non-pathogenic strains [12]. Additionally, regulatory interest in this group of pathogens continues in Canada, and risk management actions need to be supported by a comprehensive summary of the relevant information on this food safety issue. The following document has been developed to support potential risk management actions.

Scientific terminology and glossary of terms used throughout this document are defined in Table 1.

2. Purpose of the Risk Profile

This risk profile has been conducted within the Health Canada Decision Making Framework [13] and is intended to inform risk management strategies aimed at mitigating the risk of VTEC infections and the public health outcomes associated with them. The objective of this risk profile is to present a comprehensive summary of the available information on non-O157 VTEC in produce, and foods originating from cattle (e.g., beef products, milk and dairy products), to describe what is known about the potential public health threat, and to discuss the development of microbiological risk assessment and risk management strategies.

The structure of this document follows the Canadian framework [13] as well as Codex Alimentarius Commission guidelines for the development of a risk profile [14].

2.1 Scope

The following risk profile was designed to collect and synthesize current scientific information relevant to the following:

- **Hazard of concern**
  - non-O157 verotoxin-producing *Escherichia coli* (non-O157 VTEC)
- **Food products of concern**
  - Beef products, and milk and dairy products produced and consumed in Canada
  - produce (i.e., fresh fruit and vegetables) produced and consumed in Canada
- **Populations of interest**
  - Canadian population
**Term** | **Definition**
---|---
Pathogen | A micro-organism that is capable of causing disease.
Pathotype | A group of strains of a single species that cause disease with a common set of symptoms and may be defined by the possession of a specific set of virulence factors. There are six recognised pathotypes of *E. coli* which cause enteric illness (VTEC, EPEC, EIEC, ETEC, EAEC, DAEC).
Verotoxigenic *E. coli* (VTEC) | Any *E. coli* which has the genetic elements for the production of one or more verotoxins. VTEC illness can vary from uncomplicated diarrhea to hemorrhagic colitis (HC), to hemolytic uremic syndrome (HUS).
Enterohemorrhagic *E. coli* (EHEC) | VTEC which possess the locus of enterocyte effacement (LEE) pathogenicity island and induce attaching and effacing (A/E) lesions in the colon. Infection is associated with hemorrhagic colitis (HC) and hemolytic uremic syndrome (HUS). The term is also used to designate LEE negative VTEC strains which cause HC and HUS.
*Enteropathogenic* *E. coli* (EPEC) | EPEC adhere to the enterocytes of the small bowel forming A/E lesions. The primary virulence factor is the LEE pathogenicity island.
*Enteroinvasive* *E. coli* (EIEC) | EIEC cause an invasive infection of colonic epithelial cells.
*Enterotoxigenic* *E. coli* (ETEC) | ETEC adhere to small bowel enterocytes and induce watery diarrhoea by the production of heat-labile (LT) and/or heat-stable (ST) enterotoxins.
*Enteroaggregative* *E. coli* (EAEC) | EAEC adhere to small and large bowel epithelia in a thick biofilm and produce secretory enterotoxins and cytotoxins.
Diffuse-adherent *E. coli* (DAEC) | DAEC induce small bowel enterocytes to form long finger-like cellular projections, which wrap around the bacteria.
Serotype | An antigenically distinct variety within a bacterial species. The serotype of *E. coli* strains is determined by the O-polysaccharide of the lipopolysaccharide (O), the flagellin protein (H) and sometimes capsular antigens (K). Serotype may indicate a clonal relationship with a previously reported pathogen, but cannot be used to predict the pathogenicity of an isolate.
*E. coli* O157:H7 | A specific *E. coli* serotype.
*espP* | Serine protease gene.
*katP* | Catalase gene.
EtpD | Gene encoding production of hemolysin.
*hly* | Type II secretion protein.
Serogroup or O-group | An antigenically distinct variety of serotype, based only on O antigens.
Strain | A population of bacteria that is genetically homogenous, indicating clonal descent from a recent common ancestor.
O rough (OR) | *E. coli* strains which do not express O-polysaccharide or express a truncated form of the polysaccharide and thus do not react to O-antibodies.
O untypeable | *E. coli* strains which express O-polysaccharide but which are not typeable with existing O-antibodies schemes.
Virulence/pathogenicity factor | Genes which have been identified as playing role in causing illness through a specific cellular mechanism.
Putative virulence factor | Genes whose presence is associated with illness, but whose role in cellular mechanis of pathogenicity is unknown.
Pathogenicity island (PAI) | A group of genes encoding a variety of virulence factors located on a mobile genetic element.
Verotoxin island (PAI) | A toxin which kills target cells by inhibition of protein synthesis. Synonyms: shiga toxin, shiga like toxin. There are two primary variants of the toxin, VT-1 and VT-2. The genes are designated *stx1* and *stx2* (or *stx1* and *stx2*).
*stx*-phage | Group of bacteriophages which carry the genes encoding verotoxin.
Attaching and Effacing (A/E) Locus | The site of attachment of LEE expressing *E. coli*, characterised by the formation of a cellular ‘pedestal.’
Locus of enterocyte effacement (LEE) PAI | A PAI which encodes the genes required for the formation of A/E lesions.
*eae* | Gene encoding the *E. coli* surface protein intimin. *eae* is a key gene in LEE PAI and is often used to detect the presence of the LEE.
Type Three Secretion System (T3SS) | A complex protein structure used by some pathogens to inject proteins into host cells.
Produce | Means any fresh fruits and fresh vegetables (C.R.C., c.285, 2013)

**Table 1.** Summary of scientific terms and expressions used throughout this risk profile.
3. Hazard-food commodity combination(s) of concern

3.1 Hazard of concern

The hazard of concern is non-O157 VTEC. Non-O157 VTEC have been linked to many outbreaks and sporadic cases of human illness worldwide. *E. coli* is a Gram-negative rod which belongs to the family *Enterobacteriaceae*. It is facultatively anaerobic, oxidase-negative and catalase-positive, and is usually motile. The main ecological niche of *E. coli* is the gastrointestinal tract of humans and warm-blooded animals, where it appears ubiquitously [11]. There are a number of different enteropathogenic groups of *E. coli*. The six currently recognized pathotypes associated with gastrointestinal infections are enteropathogenic *E. coli* (EPEC), enteroinvasive *E. coli* (EIEC), enterotoxigenic *E. coli* (ETEC), enterohaemagglutinating *E. coli* (EAgEC), diffuse-adherent *E. coli* (DAEC), and verotoxin-producing *E. coli* (VTEC) [11]. The common feature which defines VTEC is their ability to produce verotoxins (VT) which have a distinctive cytotoxicity against Vero cells [1].

At this time, epidemiological surveillance data characterizing non-O157 serotypes show that the hazard of concern (if identified by serotype) may be different depending on population, geography, time and perhaps even food commodity. Data collected by the European enteric illness surveillance network, Enter-Net, indicate that worldwide distribution of VTEC serotypes is not uniform [15].

A study of 2852 clinical VTEC isolates collected from 2000 to 2010 reported that the most commonly reported non-O157 VTEC serogroups in the United States of America (USA) are O26, O45, O103, O111, O121, and O145 [16]. In fact, these 6 O groups have been described by the CDC as the cause of 83% of non-O157 VTEC illnesses in the USA [16]. According to an European Food Safety Authority (EFSA) report [17], the predominant non-O157 VTEC serogroups reported in the European Union are O26, O91, O103, O111, O128 and O145. These six serogroups were responsible for approximately 63% of over 2000 typeable non-O157 VTEC confirmed cases reported from 2007 to 2010 [17]. Within the EFSA surveillance report, over 4000 confirmed VTEC cases were detected where the O antigen was untyped, untypeable or reported as unknown.

In Canada, the Public Health Agency of Canada (PHAC) and provincial/territorial health authorities do not systematically test clinical samples for non-O157 VTEC. “Verotoxigenic *E. coli* infection”, including *E. coli* O157:H7 became a nationally reportable disease in 1990 [18]. Cumulative Canadian data from 1998 to 2012, based on 498 VTEC isolates reported to the Public Health Agency of Canada’s National Microbiology Laboratory (PHAC - NML), show that the commonly reported serogroups of non-O157 VTEC with confirmed VT producing status, isolated from clinical samples, include O26, O103, O111, O121, and O145 (NML, unpublished data). These six serogroups constituted approximately 52% of 498 non-O157 *E. coli* isolates with confirmed VT status (Table 2), and a further 10.8% of isolates had no O-type due to a rough phenotype or untypeable antigen (Table 3).

It is noteworthy that the goal of the above discussion (and Table 2) is to demonstrate that the worldwide distribution of serogroups and serotypes reported is not uniform. The data collected and presented for each country/union may have been collected using differing methods and over different time periods. Additionally, the choice to report the six most common serogroups associated with serious illness in this manuscript was based on convenience, given that in the USA, the sixth serogroup was identified as a cut off for serogroup listing as long ago as 2005 [19]. One challenge associated with this cut off is that the distinction between serotypes around the threshold may not be large. For example, O117 is the sixth most common serotype in Canada and is associated with 2.2% of reported isolates (11 isolates, Table 3) while serotype O91 is the seventh most common, and is associated with 2% of isolates (10 isolates). The 2007-2010 data published by EFSA [17] indicates a similar issue, where the sixth through eighth most common serotypes outside of O157 each represent 1.4% of total cases.

<table>
<thead>
<tr>
<th>Country</th>
<th>Most commonly reported non-O157 VTEC serogroups</th>
<th>% of total non-O157 VTEC illness due to six serogroups</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>U.S.</td>
<td>O26, O103, O111, O121, O45, O145</td>
<td>83</td>
<td>[16]</td>
</tr>
<tr>
<td>E.U.</td>
<td>O26, O91, O103, O111, O128, O145</td>
<td>63</td>
<td>[17]</td>
</tr>
<tr>
<td>Canada</td>
<td>O26, O103, O111, O117, O121, O145</td>
<td>51.8</td>
<td>NML, unpublished data*</td>
</tr>
</tbody>
</table>

*This estimate is based on the subset of laboratory isolations within each province reported to the PHAC and may not reflect the incidence of disease either provincially or nationally. The majority of isolates reported, originate from clinical samples, but also include food isolates associated with cases of illness.

Table 2. Most commonly reported non-O157 VTEC serogroups among clinical isolates in the U.S., the E.U. and Canada
O-Type No. of strains per O-type % of total
O26 70 14.0
O121 62 12.4
O103 55 11.0
Rough (38) or Untypable (16) 54 10.8
O111 44 8.8
O145 16 3.2
O117 11 2.2
O91 10 2.0
O5, O146, O165 9 1.8
O174 8 1.6
O8 7 1.4
O1, O113 6 1.2
O6, O48, O55, O118, O128 5 1.0
O2, O45, O69, O83, O153, O156, O177, O181 4 1.8
O43, O71, O76, O104, O119, O130 3 0.6
O28, O73, O84, O107, O110, O123, O139, O154, O179, O185 2 0.4
O116, O136, O141, O171, O18, O182, O183, O98, O21, O22, O38, O39, O41, O49, O51, O68, O70, O75, O79, O88, O4, O40, O52, O63, O78, O186, Inactive 1 0.2

Table 3. The number of E. coli isolates with confirmed verotoxin status, by O-type (excluding O157), submitted for characterization between 1998 and 2012 to the National Microbiology Laboratory, Winnipeg, MB, Canada (NML, unpublished data)

3.2 Food products of concern

VTEC infection in humans requires the ingestion of contaminated food or water, however, VTEC infection has also been documented via person-to-person contact as well as person-to-animal contact. VTEC are primarily regarded as foodborne pathogens and numerous outbreaks have been attributed to the consumption of VTEC contaminated food products [20]. As with E. coli O157: H7, beef and dairy are likely major sources for human infection with non-O157 VTEC [5]. Examples of disease outbreaks caused by non-O157 VTEC and linked to beef and dairy are presented in Tables 4 and 5. Produce has also been involved in outbreaks of non-O157 VTEC (Table 6). It should be noted that these Tables may not contain a comprehensive list of all global outbreaks.

Worldwide, the majority of data on illnesses caused by non-O157 VTEC are from foodborne outbreaks [12]. In general, sporadic illnesses caused by non-O157 VTEC are not captured and reported with enough detail to identify a food product. Given this limitation, foods associated with outbreaks of non-O157 VTEC illness include: sausage, ice cream, milk, sprouts and lettuce. In the USA, the main foods associated with human illnesses have been salads, berries, milk and beverages (e.g., cider and punch). In Canada, only one outbreak due to non-O157 VTEC has been reported, and it was associated with ground beef caused by E. coli O111:NM (Table 4) [21]. Due to the association of non-O157 VTEC with foodborne outbreaks (Tables 4-6), the broad food categories of beef products, milk and dairy products, and produce, will be the main ones covered in this risk profile.

4. Description of the public health problem

4.1 Key virulence factors contributing to non-O157 VTEC pathogenicity

The presence of known and putative virulence factors varies among non-O157 strains and likely influences their pathogenicity [22]. The virulence factor common to all clinical VTEC isolates is VT [5, 9, 23]. The VT family is divided into two immunologically distinct types: VT-1, which has >99% amino acid sequence homology with the shiga toxin of Shigella dysenteriae; and VT-2 which has a homology of only 57-60% with shiga toxin [24]. Both VT-1 and VT-2 can be divided into subtypes on the basis of amino acid sequence [24-25]. However, the production of VT cannot be solely responsible for the full pathogenicity of these bacteria, because VT-producing E. coli have been isolated from asymptomatic individuals [26]. It should be noted that VT is encoded chromosomally within a mobile genetic element, an integrated stx-phage. Thus, there exists the potential for the emergence of novel VTEC strains as a consequence of infection of other E. coli strains with the stx-phage.

Increasing evidence suggests that differences in pathogenicity among non-O157 VTEC strains are due to virulence factors encoded on mobile genetic elements [27]. The locus of enterocyte effacement (LEE) is a PAI that has been found to be frequently associated with isolates from HUS patients and outbreaks [28]. The gene eae, located in the LEE, encodes an intimin believed to mediate attachment and effacement activity required for enterohemorrhagic E. coli (EHEC, see Table 1)
colonization of the intestinal tract. Clinical evidence suggests that the presence of both eae and vtx2 in a given strain seems to increase the pathogenic potential of the strain [29].

It must be noted that, although the presence of eae has a strong statistical association with strains that have caused outbreaks and HUS [30], some LEE-negative VTEC serotypes have been the cause of serious outbreaks. A high rate of HUS was observed in the 2011 German outbreak of E. coli O104:H4 [31]. The O104:H4 strain was VT-2 positive and LEE negative and carried a plasmid that encoded attachment genes identified as virulence factors of enteroaggregative E. coli (EAEC) [32]. Sequencing of this strain indicated that it probably originated as an EAEC which was infected with the stx-phage. The outbreak and severe illness caused by this strain illustrated the potential for novel VTEC pathogens to emerge as a result of horizontal gene transfer.

The precise contribution of other putative virulence factors, many of which are located on mobile genetic elements to VTEC virulence and fitness, is unknown [33]. Other toxins purported to increase virulence include the cytotoxin subtilase (subA) [34-36], a lymphocyte-activating factor (lifA) [37], and a heat-stable toxin (astA) [38]. Some non-O157 VTEC strains contain a highly

<table>
<thead>
<tr>
<th>Year</th>
<th>Country</th>
<th>Serotype</th>
<th>Implicated Food</th>
<th># Cases (deaths)§</th>
<th>Source Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1995</td>
<td>Australia</td>
<td>O111:NM</td>
<td>Beef sausage</td>
<td>23 (1)</td>
<td>[159]</td>
</tr>
<tr>
<td>1999</td>
<td>USA</td>
<td>O111:NM</td>
<td>Ground beef</td>
<td>24</td>
<td>[21]</td>
</tr>
<tr>
<td>2000</td>
<td>Germany</td>
<td>O26:H11</td>
<td>Beef sausage</td>
<td>11</td>
<td>[160]</td>
</tr>
<tr>
<td>2004</td>
<td>Canada</td>
<td>O111:NM</td>
<td>Ground beef</td>
<td>2 (0)</td>
<td>[21]</td>
</tr>
<tr>
<td>2006</td>
<td>Norway</td>
<td>O103</td>
<td>Minced beef / Sausage</td>
<td>17</td>
<td>[161]</td>
</tr>
<tr>
<td>2007</td>
<td>Denmark</td>
<td>O26:H11</td>
<td>Beef sausage</td>
<td>20 (0)</td>
<td>[162]</td>
</tr>
<tr>
<td>2007</td>
<td>USA</td>
<td>O111</td>
<td>Ground beef</td>
<td>23</td>
<td>[21]</td>
</tr>
<tr>
<td>2009</td>
<td>France</td>
<td>O123:H-</td>
<td>Ground beef</td>
<td>2 (0)</td>
<td>[163]</td>
</tr>
<tr>
<td>2010</td>
<td>USA</td>
<td>O26</td>
<td>Ground beef</td>
<td>3</td>
<td>[164]</td>
</tr>
<tr>
<td>2010</td>
<td>USA</td>
<td>O45</td>
<td>Smoked meat</td>
<td>7</td>
<td>[164]</td>
</tr>
<tr>
<td>2011</td>
<td>Japan</td>
<td>O111</td>
<td>Raw beef</td>
<td>181</td>
<td>[165]</td>
</tr>
<tr>
<td>2013</td>
<td>USA</td>
<td>O121:H19</td>
<td>Ground beef</td>
<td>10</td>
<td>[21]</td>
</tr>
</tbody>
</table>

Table 4. Examples of non-O157 VTEC outbreaks in beef where bacteria were typed [21]

<table>
<thead>
<tr>
<th>Year</th>
<th>Country</th>
<th>Serotype</th>
<th>Implicated Food</th>
<th># Cases (deaths)§</th>
<th>Source Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1983</td>
<td>USA</td>
<td>O27:H20</td>
<td>Cheese</td>
<td>45</td>
<td>[166]</td>
</tr>
<tr>
<td>1994</td>
<td>USA</td>
<td>O104:H21</td>
<td>Pasteurized milk</td>
<td>18 (0)</td>
<td>[82]</td>
</tr>
<tr>
<td>1994</td>
<td>France</td>
<td>O103</td>
<td>Raw goat milk</td>
<td>4 (0)</td>
<td>[167]</td>
</tr>
<tr>
<td>2005</td>
<td>Italy</td>
<td>O26</td>
<td>Milk products</td>
<td>6 (1)</td>
<td>[168]</td>
</tr>
<tr>
<td>2005</td>
<td>UK</td>
<td>O26:NM</td>
<td>Cheese unpasteurized</td>
<td>6</td>
<td>[21]</td>
</tr>
<tr>
<td>2006</td>
<td>Italy</td>
<td>O92:H33</td>
<td>Cheese unpasteurized</td>
<td>14</td>
<td>[169]</td>
</tr>
<tr>
<td>2007</td>
<td>Belgium</td>
<td>O145 O26:H11</td>
<td>Ice cream</td>
<td>12 (0)</td>
<td>[170]</td>
</tr>
<tr>
<td>2007</td>
<td>USA</td>
<td>O26</td>
<td>Cheese pasteurized</td>
<td>135</td>
<td>[164]</td>
</tr>
<tr>
<td>2009</td>
<td>USA</td>
<td>O121</td>
<td>Unpasteurized milk</td>
<td>3 (0)</td>
<td>[164]</td>
</tr>
<tr>
<td>2010</td>
<td>Germany</td>
<td>O26</td>
<td>Cheese unpasteurized</td>
<td>4</td>
<td>[21]</td>
</tr>
<tr>
<td>2010</td>
<td>USA</td>
<td>O26:H11</td>
<td>Unpasteurized milk</td>
<td>6</td>
<td>[164]</td>
</tr>
<tr>
<td>2013</td>
<td>USA</td>
<td>O103</td>
<td>Milk (likely unpasteurized)</td>
<td>5</td>
<td>[171]</td>
</tr>
</tbody>
</table>

Table 5. Examples of non-O157 VTEC outbreaks in milk and dairy** where bacteria were typed [21]

† Deaths are noted in parentheses, if reported. Where no number in parentheses appears in this Table, there may not have been a reported death, or the level of detail to determine if there were fatalities may have been missing.

‡ “In beef” in this context means that epidemiological analysis has linked, but not necessarily confirmed the implication of the food listed in this Table under the column “Implicated food”. Other foods may also have been linked to the illnesses counted.

§ Deaths are noted in parentheses, if reported. Where no number in parentheses appears in this Table, there may not have been a reported death, or the level of detail to determine if there were fatalities may have been missing.

** “In milk and dairy” in this context means that epidemiological analysis has linked, but not necessarily confirmed the implication of the food listed in this Table under the column “Implicated food”. Other foods may also have been linked to the illnesses counted.
conserved plasmid (pO157), characteristic of the O157:H7 serotype, which contains genes (espP, katP, etpD, and hylA) that encode potential virulence factors [5]. The diversity in the range and types of virulence factors associated with VTEC infections in humans makes defining a single set of virulence traits difficult [12]. Elucidation of the molecular mechanisms of VTEC pathogenicity and the role of host factors has been complicated by the absence of a suitable animal model for human VTEC illness.

4.2 Characteristics of the disease

In general, the characteristics of disease caused by non-O157 VTEC are not likely to be differentiated by clinicians from those caused by E. coli O157:H7. Thus, the reported characteristics that describe clinical illness in the following paragraph are applicable in a spectrum of cases caused by VTEC, and not specific to a particular serogroup.

The onset of illness generally begins within 3 to 9 days of exposure. The onset of illness is marked by 1-2 days of stomach cramps and diarrhea; this can be followed by bloody diarrhea, if illness progresses to hemorrhagic colitis. Additional symptoms may include nausea, vomiting, fever, chills and headache. The majority of cases self-resolve, but 5-15% of cases can progress to life threatening hemolytic-uremic syndrome (HUS) or thrombotic thrombocytopenic purpura [39-40]. In adults, death may result in up to 12% of HUS cases and 25% of survivors suffer from long term health problems [41].

4.3 Susceptible populations

Infants (<1 year), children (<10 years according to the WHO, however, in Canada children are identified as <5 years) and the elderly (>65 years) are generally more susceptible to gastrointestinal illness [5]. VTEC illness with a higher rate of progression to severe outcomes such as HUS has the highest incidence in infants, young children and the elderly; even though this complication can occur in all age groups [39]. A recent surveillance study of non-O157 VTEC infections in the USA between 2000 and 2010 reported that the median age of ill persons ranged from 9 to 29 years depending on the O serogroup [16].

<table>
<thead>
<tr>
<th>Year</th>
<th>Country</th>
<th>Serotype</th>
<th>Implicated Food</th>
<th># Cases (deaths)*</th>
<th>Source Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1993</td>
<td>USA†</td>
<td>O6:NM</td>
<td>Salad / Tabouleh salad</td>
<td>160+</td>
<td>[172]</td>
</tr>
<tr>
<td>1994</td>
<td>USA</td>
<td>O153:H45</td>
<td>Potato and others</td>
<td>205</td>
<td>[173]</td>
</tr>
<tr>
<td>1996</td>
<td>USA</td>
<td>O153:H49</td>
<td>Lettuce</td>
<td>7</td>
<td>[21]</td>
</tr>
<tr>
<td>1997</td>
<td>Japan</td>
<td>O26:H11</td>
<td>Sprout, spinach</td>
<td>27 (0)</td>
<td>[174]</td>
</tr>
<tr>
<td>1997</td>
<td>USA</td>
<td>O25:NM</td>
<td>Salad (tomato, mozzarella)</td>
<td>33</td>
<td>[175]</td>
</tr>
<tr>
<td>1997</td>
<td>USA</td>
<td>O169:H41</td>
<td>Fresh vegetables</td>
<td>15</td>
<td>[175]</td>
</tr>
<tr>
<td>1998</td>
<td>USA</td>
<td>O6:H16</td>
<td>Parsley</td>
<td>66</td>
<td>[175]</td>
</tr>
<tr>
<td>1999</td>
<td>Japan</td>
<td>O188:H2</td>
<td>Salad</td>
<td>126</td>
<td>[21]</td>
</tr>
<tr>
<td>1999</td>
<td>USA</td>
<td>O111:H8</td>
<td>Salad bar</td>
<td>55 (0)</td>
<td>[176]</td>
</tr>
<tr>
<td>1999</td>
<td>USA</td>
<td>O153:H50</td>
<td>Romaine lettuce</td>
<td>40</td>
<td>[21]</td>
</tr>
<tr>
<td>2000</td>
<td>USA</td>
<td>O103</td>
<td>Punch</td>
<td>18 (0)</td>
<td>[19]</td>
</tr>
<tr>
<td>2000</td>
<td>USA</td>
<td>O169:H41</td>
<td>Basil</td>
<td>100</td>
<td>[175]</td>
</tr>
<tr>
<td>2003</td>
<td>USA</td>
<td>O169:H41</td>
<td>Coleslaw</td>
<td>41</td>
<td>[175]</td>
</tr>
<tr>
<td>2004</td>
<td>USA</td>
<td>O111</td>
<td>Apple juice unpasteurized</td>
<td>213</td>
<td>[177]</td>
</tr>
<tr>
<td>2006</td>
<td>USA</td>
<td>O121</td>
<td>Salad</td>
<td>3</td>
<td>[164]</td>
</tr>
<tr>
<td>2006</td>
<td>USA</td>
<td>O121:H19</td>
<td>Lettuce based salads</td>
<td>73 (0)</td>
<td>[21]</td>
</tr>
<tr>
<td>2006</td>
<td>USA</td>
<td>O26:H11</td>
<td>Strawberries, blueberries</td>
<td>5</td>
<td>[164]</td>
</tr>
<tr>
<td>2010</td>
<td>USA</td>
<td>O145</td>
<td>Romaine lettuce</td>
<td>31</td>
<td>[164]</td>
</tr>
<tr>
<td>2011</td>
<td>USA</td>
<td>O6:H16</td>
<td>Spinach</td>
<td>19</td>
<td>[164]</td>
</tr>
<tr>
<td>2011</td>
<td>USA</td>
<td>O26</td>
<td>Clover sprouts</td>
<td>29</td>
<td>[164]</td>
</tr>
<tr>
<td>2011</td>
<td>Denmark</td>
<td>O27:H30</td>
<td>Peas</td>
<td>87</td>
<td>[178]</td>
</tr>
<tr>
<td>2012</td>
<td>Korea</td>
<td>O169</td>
<td>Radish (kimchi)</td>
<td>1642</td>
<td>[179]</td>
</tr>
</tbody>
</table>

Table 6. Examples of non-O157 VTEC outbreaks in produce where bacteria were typed [21]

* Deaths are noted in parentheses, if reported. Where no number in parentheses appears in this Table, there may not have been a reported death, or the level of detail to determine if there were fatalities may have been missing.
† Two outbreaks from two different states were reported with the same type, trace back did not identify a single source.
‡ “In produce” in this context means that epidemiological analysis has linked, but not necessarily confirmed the implication of the food listed in this Table under the column “Implicated food”. Other foods may also have been linked to the illnesses counted.
Two surveillance studies conducted in Canada have identified the median and mean ages of patients identified with non-O157 VTEC. A surveillance study conducted in the province of Manitoba, 2002-2004, reported the age of individuals from whom non-O157 VTEC were isolated, ranged from 8 months to 87 years, with the mean and median ages being 30 and 22 years of age, respectively [7]. The goal of this study was to systematically detect and isolate O157:H7 and non-O157 VTEC from patients suffering from diarrheal illness or HUS, and in total, it identified 32 isolates of non-O157 VTEC [7]. A study conducted in Alberta, Yukon, the Northwest Territories, and Nunavut, between 2007 and 2008 reported that the median age of 14 VTEC patients identified (12 non-O157, 2 O157:H7), was 21 years [9].

There are challenges with the notion, however, that a susceptible population can be easily or strictly defined. Recent outbreaks of illness of non-O157 VTEC have demonstrated that the definition of a vulnerable or susceptible population could vary depending on the pathotype or serotype, as well as other factors, for instance the food which is contaminated and typical consumption patterns of that food. As a point of reference, healthy adults (20-65 years) formed the vast majority of cases in the 2011 VTEC O104:H4 outbreak in Europe associated with sprouts of fenugreek seeds [42]. According to the reported epidemiological data for this VTEC outbreak, the median age of patients with HUS was 43 years old and 89 % of HUS cases were adults [43] with a predominance of women (68% of HUS cases).

4.4 Estimating annual incidence rate

VTEC infection has been notifiable in Canada since 1990, and it is subject to national reporting by the Public Health Agency of Canada (PHAC). However, the data available is limited due to the absence at the clinical level, of routine testing for VTEC other than E. coli O157:H7. Data from the National Enteric Surveillance Program (NESP) within PHAC indicates that 1.39 Canadians per 100,000 were infected with E. coli O157:H7 in 2011 [44]. The annual incidence rate of non-O157 VTEC in Canada has not been estimated by the National Enteric Surveillance Program. Table 2 summarizes the number of reported clinical isolates of VTEC in Canada between 1998 and 2012 (NML, unpublished data). Because of significant under reporting and lack of a systematic national surveillance program, these figures only represent a subset of laboratory isolations within each province, and may not accurately reflect the incidence of disease [45].

A recent estimate, incorporating corrections for under reporting, proposed that foodborne E. coli O157:H7 infections may occur at a rate of approximately 39 per 100,000 in Canada [46]. The same study estimated that in Canada, non-O157 VTEC infections may occur at a rate of approximately 63 cases per 100,000 [46].

The challenge in determining the true incidence of non-O157 VTEC infection is not unique to Canada. The reported cases of foodborne outbreaks of non-O157 VTEC shown in Tables 3, 4 and 5 may not constitute a representative picture of clinical frequency as they exclude sporadic cases. Estimates of the rates of VTEC illness have evolved over time in other jurisdictions. Mead et al. [47], estimated 93,787 cases of foodborne VTEC infection each year in the USA, of which 67% are attributed to E. coli O157:H7. A more recent estimate is 175,905 cases of foodborne VTEC illness each year in the USA, of which 36% are attributed to E. coli O157:H7 [48]. This latter figure is in much better agreement with the results of the FoodNet surveillance program [6]. In Europe, the estimated average number of confirmed cases of O157:H7 and non-O157 per annum between 2007 and 2010 were 85,222 and 149,445, respectively [49].

4.5 Outcome of exposure

It has been established that certain types of non-O157 VTEC can cause illness with outcomes that are comparable to E. coli O157:H7 [22]. However, the actual threat posed by individual strains of non-O157 VTEC is highly variable. Some strains appear to be associated with sporadic and not outbreak cases, and to cause only relatively mild self-limiting diarrhea or asymptomatic infection.

As of the writing of this paper, there has only been one foodborne outbreak associated with non-O157 VTEC recorded in Canada (Table 4). This outbreak (2004) involved two cases associated with VTEC O111:NM in ground beef [21]. Aside from this outbreak, there are reports of single cases of illness caused by non-O157 VTEC, but the linkage between cases and food sources is difficult. There is a scarcity of data for severe outcomes (hospitalisation, long term sequelae, and death) as a result of foodborne infection with non-O157 VTEC in Canada. This is due to the limited number of reported isolates, combined with difficulties in identifying epidemiological links between sporadic cases and food products [50].

Clinically, VTEC infection is a major health concern because of its propensity to cause HC and HUS [51]. In the previously referenced E. coli O104:H4 outbreak in Europe in 2011, there were 4075 confirmed cases of illness and 50 deaths [4]. The severity of illness in this outbreak was high, with more than 20% of the 4075 confirmed cases progressing to HUS [31]. One travel-related case linked to this outbreak was reported in Canada [42]. In North America, approximately 20% of HUS cases caused by VTEC may be associated with non-O157 VTEC [52].
4.6 Long-term sequelae

Pathogenic non-O157 VTEC serogroups can cause life-threatening disease. The serious adverse health effects referred to previously, which are caused by these organisms, include the following:

- **HUS** is defined by three features including acute renal failure, thrombocytopenia, and microangiopathic hemolytic anemia [43]. In a prospective study of exposure to VTEC among Canadian children with HUS [53], it was reported that HUS is a leading cause of acute renal failure in children. In general, children with HUS suffer long-term sequelae, including arterial hypertension [54]. Other important sequelae seen in patients with HUS include: neurological injury, pancreatitis, seizures, and blindness [55].

- Non-HUS cases are generally characterized by abdominal pain [43] and liquid or bloody diarrhea [9, 32, 43], and are not generally associated with frequent or severe long-term complications.

4.7 Treatment

No specific therapeutic interventions are available for HUS in humans [28, 56]. Patients with severe VTEC disease and HUS require diligent monitoring of urine output, weight, volume status, cardiovascular/respiratory function and other organ involvement [57]. Patients may present with a number of symptoms as a result of dehydration and poor kidney function that may require renal replacement therapy (dialysis).

Although antimicrobial resistance by pathogens is generally considered a factor which leads to greater human health risk as a result of potential treatment failure [58], it may not be relevant to the treatment of VTEC illness. Administration of antibiotic therapy to treat VTEC infection is controversial. Several experts advise against the use of antibiotics for the treatment of VTEC infections, since antibiotic therapy can be regarded as harmful in the management of HUS [51]. However, aggressive use of antibiotics for the treatment of HUS in the 2011 European *E. coli* O104:H4 outbreak was not reported as harmful, and might have improved clinical outcomes [59].

4.8 Epidemiology of foodborne disease

Surveillance studies conducted on patients with diarrheal illness provided evidence of a significant rate of sporadic cases of non-O157 VTEC infection in Canada. A Manitoba study reported that 63% of 32 VTEC isolates recovered from patients from rural areas were non-O157 VTEC and were not linked to other cases [7]. A surveillance study of 2,328 stool samples from diarrheal patients in Northern Alberta in 2009-2010, reported 13 non-O157 VTEC and 8 O157:H7 VTEC that also were not linked to other illnesses. A similar study of 2,702 stool samples from Alberta, Yukon, the Northwest Territories, and Nunavut, reported that 38 samples were positive for VT genes and of 15 isolates recovered; there were two *E. coli* O157:H7, one *E. coli* O157:H16 and 12 non-O157 VTEC [9].

The NESP annual reports document the epidemiological characteristics (e.g., PFGE profile, number of cases, suspected source, outbreak type, etc.) of *E. coli* O157:H7 outbreaks in Canada. However, there is a lack of epidemiologic and source attribution data from non-O157 VTEC foodborne illnesses. The lack of source attribution may be due to the limited number of reported non-O157 isolates, combined with the difficulties in separating possible true non-O157 illness or outbreak events from chance associations [50].

Available studies have noted seasonal variations in the occurrence of human VTEC illness. For instance, a prospective study of cases of sporadic diarrhea due to VTEC from July 1984 to June 1986 [60], reported that 36 (0.7%) of 5,415 stool specimens from patients in Alberta contained non-O157 VTEC. The same study showed that both VTEC O157:H7 and non-O157 VTEC, outbreak and sporadic cases, typically peaked in the summer months [60].

4.9 Economic impact or burden of the disease

The costs of medical and productivity loss of a 1995 outbreak of *E. coli* O111 in Australia were estimated at $5.6 million AUS [61]. This outbreak was caused by contaminated Mettwurst, and the result was approximately 200 cases; 23 cases of HUS and one death. In the USA, the cost for each case of non-O157 VTEC infection is estimated to be $1,764 [62].

In Canada, on average, people suffer 1.1-1.3 episodes of infectious gastrointestinal illnesses per year, with associated costs estimated at $115-129 CAD per capita and an average of $91 CAD per case [63-65]. However, there is no peer-reviewed and published information on the adverse economic consequences (hospitalisation, cost of medications and working days lost) due to non-O157 VTEC foodborne illnesses.

5. Food production, processing, distribution and consumption

An understanding of microbiological, environmental, farm, processing and food handling factors are needed to perform a risk assessment, as well as to develop evidence-based policies. In a recent review concerning the significance of non-O157 VTEC in food, it was proposed that the contributing factors to the health risks from VTEC associated with the consumption of food products...
include: the virulence of contaminating strains or pathotypes, the initial contamination levels, and the survival of these organisms during the food processing and preparation steps [12]. The sections below focus on the potential for initial contamination and the survival of VTEC during the food processing continuum of beef, milk and dairy in Canada.

With respect to food consumption, the Canadian Community Health Surveys provide information on the amounts of various foods consumed in a serving in Canada. Additionally the amount of beef, milk and dairy as commodities, available to Canadians for consumption, both domestic and imported, was investigated for this risk profile.

The majority of beef, milk and dairy consumed in Canada are of domestic origin. Imported fluid milk represented 1% of fluid milk by volume consumed by Canadians (2011 data), and imported beef represented 18% of beef by weight consumed by Canadians (2012 data) (Agriculture and Agri-food Canada, personal communication) [66]. In contrast, domestic fresh produce (i.e., fruits and vegetables, not including potatoes) available for consumption in Canada is generally outweighed significantly by imported fresh produce. For example, imported fruits and vegetables represented approximately 90% and 75% respectively of the produce that was available for Canadian consumption in 2010 (Agriculture and Agri-food Canada, personal communication). This is attributed to the seasonal nature of fruit and vegetable production in Canada.

The details of primary food processing for imports, especially in the case of fruits and vegetables, are not fully represented in the following sections because practices and regulations can vary outside of Canada. It is understood that each country may have its own protocol for on-and off-farm food safety requirements, as well as its own food safety standards and regulatory requirements.

5.1 Beef products

Ruminants, including cattle, are hosts to both E. coli O157:H7 and non-O157 VTEC [5, 23, 67]. Fecal shedding of VTEC by ruminants is believed to be the primary origin of human infection with both O157:H7 and non-O157 VTEC [5].

Cattle hides have been identified as a major source of contamination of beef carcasses with non-O157 VTEC [68]. Muscle tissue is essentially sterile, but carcass surfaces can become contaminated with microorganisms during slaughter. The microbial contamination originating on animal hides can potentially transfer to the carcass, survive decontamination and further, can multiply on carcasses and production surfaces during subsequent processing stages. It has been proposed that there is a positive correlation between carcass contamination and pre-harvest carriage of E. coli O157:H7 [69], but this hypothesis is contradicted by the findings of a study of hide and pre-evisceration carcass contamination by E. coli O157:H7 and Salmonella at four slaughter plants, that found that the prevalence of pathogens on carcasses was dependent upon the plant (presumably plant practices) and independent of the hide prevalence [70]. Similarly, fecal shedding of O157:H7 and non-O157 VTEC by cattle is higher in warmer months of the year but there is no clear indication that this results in increased carcass contamination. A study at three slaughter plants in the USA [68] reported that the prevalence of O157:H7 and non-O157 VTEC and Salmonella on pre-evisceration carcasses was seasonal. Another study of four slaughter plants in the USA [70] reported no seasonality in the prevalence of E. coli O157:H7 and Salmonella on pre-evisceration carcasses.

A study regarding post-harvest interventions to reduce pathogens in beef, indicated that 53.9% of beef carcasses in large processing plants in the USA were positive for at least one strain of non-O157 VTEC prior to evisceration, and the prevalence was reduced to 8.3% post-slaughter by using various intervention strategies such as steam vacuum, steam pasteurisation, organic acids and hide treatments [71]. Similar data have been obtained which support this study [68]. The results of studies of pre-harvest interventions with O157:H7 show that no one method has been demonstrated to reliably prevent carriage or reduce fecal shedding of O157:H7 VTEC under commercial conditions. Additionally, experimental results can often be contradictory. Reduction in fecal shedding has not been demonstrated to reduce hide carriage and no data is available on impacts on carcass or meat contamination.

At slaughter, initial bacterial contamination of cattle carcasses can be minimised by operators following best practice in de-hiding, de-heading and evisceration [72]. Treatment of beef carcasses with steam or hot water pasteurisation, chilling with drying, as well as lactic acid spraying have all been demonstrated to effectively reduce the numbers of total E. coli under commercial conditions in Canadian beef plants [72]. In the absence of evidence that VTEC are more resistant to inactivation treatments than other E. coli, it can be presumed that they will also be reduced.

Under the next phases of processing, manipulation of beef products through such processes as tenderization, may alter bacterial contamination. In the case of mechanical tenderization, it is known that the tenderizing process can translocate pathogens from the surface to interior sites of beef [49].
With respect to other post-processing issues, Duffy et al. [73], performed a review of issues pertaining to E. coli O157:H7 and noted that the failure to maintain chill temperatures during storage and retail display, may allow growth of the pathogen. Although it is not expected that VTEC will grow at cooler temperatures, temperature abuse may affect the microbial condition of a product. Additionally, improper handling of raw meat or leakage from wrapped packages may lead to cross-contamination.

Non-O157 VTEC have been isolated from beef products in Canada, however, non-O157 VTEC illness outbreaks have only been epidemiologically linked once to the consumption of beef or beef products in Canada (Table 4). This may imply that the level of contamination or bacterial load of non-O157 VTEC may be low, however, this has yet to be confirmed. Correspondingly, the true global meat contamination rates and contribution of non-O157:H7 VTEC to human infection remains mostly unknown [74]. There is also little information on the prevalence and concentration of non-O157 VTEC on beef and beef products. It is noteworthy that available studies indicate outbreaks of non-O157 VTEC associated with beef products are commonly linked to undercooked meat products [75].

5.2 Milk and dairy products

Milk, as well as dairy products present a good medium for the growth of many spoilage and pathogenic microorganisms [76]. Milk and dairy products from cattle and ruminants are potential vehicles of VTEC and have been linked to outbreaks of both O157:H7 and non-O157 VTEC infections [77].

For dairy products, the major source of milk contamination by VTEC is faecal contamination. The prevalence of non-O157 VTEC in faeces of dairy cattle ranges from 0.4% to 74.0% [78]. Hence, avoiding faecal contamination by VTEC during milking is crucial for managing this pathogen on the farm. The implementation of effective cleaning procedures and other precautions during milking are important, because contaminated milking equipment can facilitate the spread of pathogens to bulk storage vessels [79]. In 1997, it was reported that 15 out of 1720 raw milk samples from farm bulk tanks in Ontario, Canada, were positive for non-O157 VTEC [80]. Additionally, some of the non-O157 VTEC serogroups that are most commonly identified in severe VTEC human illnesses in Canada (Table 2) have been isolated from raw milk samples. These findings are not unique to Canada and are not necessarily unexpected in an unpasteurized product. Surveillance of Irish dairy herds, also reported the presence of non-O157 VTEC in raw milk samples [81]. Non-O157 VTEC have been isolated from unpasteurized milk and dairy products in Canada, however, epidemiological association of non-O157 VTEC outbreaks linked to the consumption of milk and dairy products in Canada has not been reported (Table 5). It is noteworthy that available studies indicate outbreaks of non-O157 VTEC associated with milk and dairy products are commonly linked to unpasteurized products (Table 5). With regards to pasteurized milk products, non-O157 VTEC outbreaks are usually caused by pasteurization failure or post-pasteurization contamination. For example, an outbreak in Montana caused by VTEC O104:H21 was attributed to post-pasteurization contamination of milk [82].

5.3 Produce

Fresh produce may be susceptible to contamination with VTEC throughout the field-to-fork continuum [15]. The location of the edible part of crops during growth (underground, on the soil or above the ground), combined with produce surface characteristics (e.g., presence of cracks, crevices), and the method of processing, are all factors which may affect microbial survival in fresh produce during both on-farm and off-farm activities.

Direct means of on-farm microbial contamination include contact of produce with contaminated water or with untreated manure. These contamination routes have been cited as the two most important at the pre-harvest level with the pathogen originating from human or animal waste material [83]. For instance, both E. coli O157:H7 and non-O157 VTEC such as O26:H11, have been reported to survive in cattle manure for extensive periods, ranging from 42 days to up to four months [84 - 85]. It follows that the use of untreated manure as a fertilizer on fields can be regarded as a possible contamination route, specifically for fresh produce commodities which may have been subjected to minimal or no processing prior to their consumption.

Available data indicate that the use of contaminated well water for irrigation of fresh fruits and vegetables cannot be ruled out as a source of on-farm produce contamination with non-O157 VTEC, since it has been shown that E. coli O26:H11 and O111:H8 can survive in well water for up to 56 days at 10 °C [86]. Additionally, data from an FDA environmental assessment in response to a multistate outbreak of human E. coli O145 infections linked to Romaine lettuce, showed that contamination of an area surrounding the produce growing field may have contributed to the introduction of E. coli O145 into irrigation water, which in turn may have contaminated the produce [87].
The inner tissues of fruits and vegetables are generally regarded as sterile [88], therefore, off farm contamination can arise as a consequence of application of post-harvest treatments, such as spraying or cutting, which remove the natural protective barriers, and thus create favorable conditions for VTEC survival and growth [15]. For example, cutting practices can increase microbial levels up to 6-fold on vegetable surfaces [89]. Also, some fresh produce such as baby spinach and Romaine lettuce are difficult to render microbe free with washing [90].

Fresh fruits and vegetables are often consumed with minimal processing or are sold ready-to-eat. In these cases, the conditions of storage and transportation may likely support growth or survival of non-O157 VTEC throughout the shelf-life of the product. Additionally, multiple factors may cause contamination at both on-farm and off-farm processing steps, including (i) the use of unclean containers and tools used in packing, transporting, or processing, (ii) the lack of good worker hygiene practices, and (iii) cross-contamination from other products [91].

A wide variety of produce items have been implicated in outbreaks of non-O157 VTEC illness in humans (Table 6). The largest foodborne outbreak of non-O157 VTEC to date was linked to fenugreek sprouts in Europe in 2011 [4]. In Canada, a recent review of produce-borne outbreaks from a range of microbial pathogens from 2001 to 2009 found that leafy greens and herbs are the top two produce-borne illness routes in Canada [92]. These products accounted for 52% of all Canadian produce-related outbreaks [92]. Additionally, the FAO/WHO ranked leafy greens and herbs as the greatest microbiological hazards of concern in fresh fruits and vegetables [93].

5.4 Current risk management practices in beef, milk and dairy products, and produce

Since cattle and other domestic ruminants are known reservoirs of VTEC, it has been proposed that the environmental presence of VTEC and contamination of meat and milk from these animals, could be reduced by preventing carriage of VTEC or reducing the numbers of VTEC shed in feces or the numbers of VTEC present on animal hides presented for slaughter. A wide range of potential pre-harvest interventions for the control of E. coli O157:H7 in cattle have been investigated. The proposed interventions include, changes to animal management and transportation, diet, hygiene of feed water and bedding, feed additives, antimicrobial and bacteriophage treatments, vaccination and pre-slaughter hide washing [94 – 96]. Though the strategy of pre-harvest intervention has been proposed as having great potential [94 - 96], currently no intervention has been demonstrated to prevent VTEC carriage or to reliably reduce VTEC shedding via cow feces. It is recommended that cattle producers follow best industry practices for cattle management and hygiene of food, water and transportation.

With respect to fresh fruits and vegetables, environmental contamination particularly affecting the source of irrigation water, can lead to contamination with non-O157 VTEC in the product [87]. Completing an environmental risk assessment, e.g., the evaluation of production environment, wildlife and domestic animal activity of the area surrounding the field in which fruits and vegetables are grown, is an important step to achieving produce safety. This approach allows for the identification of potential sources of irrigation water contamination prior to and during production activities, so that potential contamination can be managed proactively. In this regard, the use of Good Agricultural Practices as described by Codex [97] could provide the basic framework to help minimize microbiological contamination of produce at any point during pre-harvest and harvest.

In Canada, guidance/guidelines for fresh produce exist, including the Canadian Food Inspection Agency’s (CFIA) Code of Practice for minimally processed ready-to-eat vegetables [98], and the Health Canada policy on sprouted seeds and beans [99]. Health Canada also recommends the testing of sprout irrigation water for the two top pathogens, i.e., Salmonella and E. coli O157:H7, responsible for 83% of all produce-borne outbreaks in Canada [92]. Currently, the microbiological testing of sprout irrigation water as per the Health Canada policy [99] does not include non-O157 VTEC.

Regardless of the food commodity, intervention-based hazard analysis and critical control points (HACCP) in the food production and processing environment is recommended as a management system for delivering microbiologically safe food [100]. Many processors currently have HACCP or GMP processes in place to control E. coli O157:H7. Since there is no evidence that non-O157 VTEC are more resistant to environmental stresses and decontamination processes currently used in food processing than E. coli O157:H7 and other E. coli, it can be reasonably expected that systems currently in place which effectively control E. coli O157:H7 and other enteric pathogens will control non-O157 VTEC. The success of such a system is dependent upon the general hygiene of the processing environment, especially where products cannot be subjected to a lethal processing treatment.

Training of personnel in food handling and preparation is another area that could improve the safety of the food
supply. Since there is evidence of asymptomatic carriers of VTEC in the population [26], screening food handlers may be relevant [101]. Consumer education on hygienic handling and adequate cooking of food can also play a role in reducing the incidence of VTEC infection. Programs aimed at consumer food safety education, such as the Canadian FightBac campaign (http://www.canfightbac.org), have been developed in partnership with Health Canada. These programs emphasize the need for frequent washing of hands and surface areas in contact with food, separation of foods during storage and preparation to avoid cross-contamination, proper cooking temperatures to kill pathogens that may be present, and prompt refrigeration of purchased foods including raw, cooked and leftover meals and products.

5.5 Resistance of non-O157 VTEC to physical and chemical inactivation

The resistance of bacteria, including *E. coli*, to physical and chemical inactivation varies between strains of the same species, and between strains of the same serotype [102 - 107]. Foods are complex physicochemical environments and the response of cells to inactivation is also dependent on the food matrix. Components such as organic acids, carbohydrates, salts, proteins, and lipids and physicochemical parameters such as pH, water activity and osmotic concentration may affect bacterial inactivation [108 - 110].

Because individual strains of *E. coli* may vary significantly in their resistance to different forms of physical and chemical inactivation, the results of studies performed with single or small numbers of strains may not be representative of all VTEC. However, a number of studies which have compared the resistance of non-O157 VTEC with that of O157:H7, have shown that conditions sufficient to inactivate the most resistant O157:H7 VTEC strains will also inactivate the tested non-O157 VTEC serogroups [103, 111 – 116].

Comparisons of the recovery of strains of different VTEC serogroups, including O157, O26, and O111, suggest that they behave similarly when stressed in foods such as minced beef (freezing/thawing), apple juice (acidification), pepperoni (salting), and cheese [117]. In addition, the growth kinetics and survival of *E. coli* O157:H7 and *E. coli* O26 during food enrichment were studied in order to compare their survival under typical processing conditions [118]. No significant difference in growth kinetics, lag phase or growth rate was observed between the O157:H7 serotype and the O26 serogroup. These VTEC strains survived for approximately 18 days in yogurt with pH ranges from 4.1 to 4.3, and approximately 30 days in orange juice (pH 4.2 to 4.5) at 4°C. Additionally, their thermal tolerance at 55 °C in minced beef was comparable.

The results of Duffy *et al.* [118] indicate that where a process (based on low pH or heat) is validated to inactivate *E. coli* O157:H7, the same conditions should also be effective against *E. coli* O26. The limited data collected by Drysdale *et al.* [117] suggest that certain non-O157 VTEC strains behave similarly to *E. coli* O157:H7 at the physiological level when challenged by food relevant conditions of temperature, pH, salt and water content.

Chlorine is the most commonly used sanitizer in the fresh produce industry [119 – 120], and there is currently no information regarding what concentration and exposure time may be necessary to inactivate non-O157 VTEC [121]. However, the evaluation of the effects of free-chlorine concentration, organic load, and exposure time on the inactivation of non-O157 VTEC belonging to the serotypes O103:H2, O26:H11, O45:H2, O111: NM and *E. coli* O157:H7, showed that 1.0 mg/liter of free-chlorine was sufficient to inactivate both non-O157 VTEC and *E. coli* O157:H7 in the wash solution with 1% of lettuce and tomato organic matter, but the extent of inhibition of these organisms was organic load-dependent [121].

The ability of VTEC biofilms to survive sanitation processes is a food safety concern [122]. In one study, biofilms were formed by 3 strains each of VTEC O157:H7, O26:H11 and O111:H8 on stainless steel surfaces and treated with either 300 ppm quaternary ammonium chloride based sanitizer or 200 ppm chlorine. Both high and low resistance to the sanitizer treatment was observed for strains of all three serotypes. Additionally, this study reported that, depending on the temperature at which the biofilms were grown, both *E. coli* O157:H7 and non-O157 VTEC biofilms were associated with a range of survival rates after exposure to quaternary ammonium chloride [123].

Most of the studies reviewed support the conclusion that O157:H7 and non-O157 VTEC respond similarly to inactivation treatments in food processing and that serotype is not a useful predictor of the potential resistance of individual strains.

Given, that *E. coli* O157:H7 and non-O157 VTEC behave similarly, the following characteristics of *E. coli* O157:H7 are relevant to this risk profile of non-O157 VTEC [124 – 125]:

- *E. coli* O157:H7 can survive for extended periods at -20 °C in food.
- The effect of pH on the bacterium is dependent on the type of acid present in its environment. For example, it can grow at pH 4.5 in medium adjusted with hydrochloric acid, but not when adjusted with lactic acid. The pH range for the growth of *E. coli* O157:H7 is 4.4 – 9.0, with the optimum pH in between 6.0-7.0.
This bacterium grows slowly in broth containing 6.5% but not 8.5% NaCl, as well as survive for long periods of time on fermented or acidic foods. The minimum water activity for growth is 0.95 and the optimum is 0.995.

6. Public perception of the problem and the risks

Public opinion research can help to identify which tools best fit public needs. To-date, there are no available data regarding the public perceptions of the problem and the health risks specifically associated with non-O157 VTECs in Canada. However, a recent survey by the Canadian Food Safety Alliance reported that E. coli contamination of food is the top food safety concern of Canadians [126].

Interestingly, epidemiologic evidence in outbreak cases of E. coli O157:H7 attributed to beef, continue to be associated with consumers and or service sectors who may not understand the risks of handling raw meat and who demonstrated inadequate handling or cooking practices [73]. This information suggests that the need to educate consumers exists, and proper handling of raw meat may be tied to public knowledge of the risks due to VTEC.

7. Summary of Available Information and Major Knowledge Gaps

7.1 Current regulatory and inspection action for non-O157 VTEC

There are no internationally agreed upon regulatory and/or inspection actions for non-O157 VTEC.

On September 20, 2011, the U.S. Department of Agriculture’s Food Safety and Inspection Service (USDA-FSIS) officially declared intimin (eae) positive VTEC strains belonging to 6 priority O-groups (O26, O45, O103, O111, O121 and O145), in addition to E. coli O157:H7, to be adulterants of non-intact raw beef products and its components [127]. The USDA began testing for these serogroups as of the 4th of June, 2012.

The European Commission regulation 2073/2005 on the microbiological criteria for food requires that E. coli be monitored as an indicator of hygienic conditions. Since VTEC strains belonging to serogroups such as O26, O103, O111, O145 and O157 often cause foodborne infections, the EFSA Biological Hazards (BIOHAZ) panel also recommended their monitoring in raw and RTE foods [128]. Additionally, bovine meat-borne pathogenic VTEC was recently categorised as a high-priority for meat inspection, however, the criteria for pathogenicity have not been fully elucidated in the European context [49].

In Canada, produce, and foods originating from cattle (e.g., raw ground beef, and dairy products) found positive for E. coli O157:H7 have defined actions associated with these results as per federal guidelines [129-130] and regulation [131]. Although current federal action as a result of a positive test result for non-O157 VTEC is not fully defined, non-O157 VTEC positives would be addressed on a case-by-case basis and all food commodities sold in Canada are governed under the Food and Drugs Act which prohibits the sale of food contaminated with a harmful substance [132].

7.2 Areas where major information gaps exist

Regardless of the risk analysis being performed, the hazard or hazards must be clearly identified. In a quantitative microbial risk assessment (QMRA) the risk posed by those particular hazards or pathogens should be calculated. In addition, the primary goal of a QMRA is often to establish an estimate of the probability and severity of illness in a given population from eating a serving of contaminated food. In more advanced analysis, there is the possibility to investigate the effectiveness of interventions and provide more specific advice to develop risk management actions. As well, a part of more advanced analysis is the opportunity to characterise and quantify the uncertainty and variability associated with the assessment of the risk and the analysis of interventions. It would be useful to perform a QMRA for non-O157 VTEC, when more data become available.

7.2.1 Data gap #1: Epidemiological linkages

In order to complete a focused risk assessment, the hazard must be clearly identified and a clear decision must be made from a risk management point of view, specifying the need to address this hazard. Epidemiological linkages through food attribution are critical for the initiation of risk analysis processes. Thus, food attribution, or the ability to attribute cases of foodborne disease to a food vehicle [133] is key in estimating the food specific burden of the illness, which in turn informs decision-making for effective prevention and control of foodborne pathogens [134-136]. There is considerably less food attribution data available for non-O157 VTEC compared to O157:H7 VTEC in Canada, although surveillance studies [7-9] indicate that the majority of VTEC illness in Canada is caused by non-O157 VTEC. Successful food attribution is dependent upon prompt identification of the causative organism and follow-up investigation, including patient history and analysis of implicated food. Follow-up investigations are typically performed only after outbreaks and only one outbreak (attributed to ground beef) involving non-O157 VTEC has been reported in Canada [21].
7.2.2 Data gap #2: Laboratory methods for detecting non-O157 VTEC in foods

Effective surveillance and epidemiological studies are reliant on effective laboratory methods for detecting the pathogen of concern. The detection and isolation of VTEC from all sample types remains a challenge due to the genotypic and phenotypic diversity of VTEC [17, 137-138]. Despite the challenges, methods for the detection and isolation of non-O157 VTEC in foods have been published in peer-reviewed journals or by regulatory agencies [139-147]. However, the conceptual and practical implementation of these methods and their capability to differentiate the important genotypic and phenotypic variations of non-O157 VTEC is not comprehensive. Specifically, the challenges associated with the culture-dependent methods include the lack of a standardised enrichment protocol [141], and difficulty distinguishing VTEC from non-pathogenic E. coli based on appearance on many of the selective and differential media [3]. VTEC have variable resistance to a range of antimicrobials [140], whereas O157 VTEC have relatively high resistance to certain antibiotics (i.e., novobiocin, potassium tellurite and cefixime) and this characteristic adds a level of complexity to the development of effective enrichment stages in the detection of non-O157 VTEC in foods [148].

Molecular methods can be used to detect the presence of virulence genes and the specific gene associated to a serogroup [141]. Targets for food testing include VT-1 and VT-2 and eae, along with markers for priority O serogroups [139, 145]. The U.S. Department of Agriculture’s Food Safety and Inspection Service (USDA-FSIS) have declared intimin (eae) positive VTEC belonging to priority serogroups O26, O45, O103, O111, O121, and O145 to be adulterants in non-intact beef products. A method has been developed to support this program. However, in the context of food safety and foodborne illness investigations, a serogroup-independent approach might be more informative. Disease-causing serogroups of non-O157 VTEC have been shown to possess multiple combinations of virulence factors and the role of some putative virulence factors is still uncertain [139]. Additionally, it is expected that horizontal gene transfer will continue to occur and new VTEC strains, pathogenic or not will continue to emerge [81].

In 2006, the Canadian Federal VTEC Network was established jointly by Health Canada, the CFIA and PHAC. The Canadian VTEC Network functions to deliver the scientific and laboratory expertise needed to support the development of VTEC detection methods, including non-O157 serotypes [149].

A method collaboratively developed by the CFIA and Health Canada has been validated for its efficacy in the detection of VTEC serogroups O26, O103, O111 and O145, as well as O157 from ground beef, leafy greens and apple juice [140]. Subsequently this method has been expanded to include the isolation and identification of serogroups O121 and O45, and validated for the detection of the USDA-FSIS six identified non-O157 VTEC serogroups (O26, O45, O103, O111, O121, and O145) plus O157 in beef trim [144]. The Canadian method is based on the enrichment of samples in a selective broth optimized for the recovery of non-O157 VTEC. The VTEC enrichment broth has been shown to support the recovery of a wide variety of VTEC [140]. Enrichment of samples is followed by PCR screening of broths targeting VT-1, VT-2 and eae (VTE-PCR). Presumptive positive samples are plated onto agar media and VTEC colonies are identified by VTE-PCR. VTEC isolates are characterized using a multiplex PCR and cloth-based hybridization array system (CHAS), also called the EHEC-7 CHAS.

In the event of the detection of isolates not falling within the serogroups targeted by the EHEC-7 CHAS, a DNA sequence-based web tool for typing of VTEC using the gnd gene (called the E. coli O-Typer) was developed. It enables prediction of the serogroup of clinical E. coli strains [150]. This molecular method was successfully used to characterize both priority VTEC (e.g., O26, O121) and non-priority pathogenic VTEC serotypes (e.g., O177:NM) during outbreak investigations prior to serological confirmation [150]. The E. coli O-Typer was also used to characterize O104:H4 clinical isolates during the 2011 E.U. produce-borne outbreak [32].

7.2.3 Data gap #3: Dose-response of non-O157 VTECs

Microbial pathogens can be introduced into the body from a variety of sources. To cause illness, a sufficient number of cells must be consumed. This is known as the dose-response and varies from one organism to another and from person-to-person [5].

Currently, the dose-response of E. coli O157:H7 (NM) for humans has not been determined by direct challenge studies, but has been estimated based on information from outbreak investigations. The infectious dose is estimated as 100-200 cells [151], and possibly as low as 10 cells [152]. This dose-response for E. coli O157:H7 is low relative to many other foodborne pathogens [153].

In contrast to E. coli O157:H7, which is strongly associated with foodborne disease outbreaks and very severe consequences of infection [154], limited data are available regarding the dose-response of non-O157 VTEC. However, as a result of molecular microbiological investigation of an outbreak of HUS caused by the
consumption of dry fermented sausage contaminated with non-O157 VTEC [155], a total E. coli O111:NM count of less than 100 CFU/g was reported in the sausage eaten by ill patients. A demonstrative dose-response relationship for E. coli O111 and O55 was previously developed using data reported from human volunteer studies [156]. In this example, the relationship for both strains combined resulted in an estimated median infectious dose (ID₅₀) of 2.6 x 1₀⁶ organisms.

These examples, however, need to be viewed with caution because of the reasons stated above and relating back to the heterogeneous nature of this group of bacteria.

7.2.4 Data gap #4: Prevalence and concentration of non-O157 VTECs in food

It has been stated that the best way to control and eliminate pathogens is to understand their source and prevalence [157]. With regards to produce and foods of cattle origin, very little information on the prevalence/incidence and concentration of non-O157 VTEC in Canada is available. Moreover, nationwide microbiological baseline data on the prevalence of non-O157 VTEC in food is not available yet. The prevalence and levels of pathogenic non-O157 VTEC in food products in Canada should be determined to provide an estimate of the food specific burden of illness, and to set priorities for data collection for the prevention and control of these pathogens. Mathematical modeling could possibly allow us to assess the effect of microbiological controls and also help to define an appropriate sampling plan at critical control points during production.

8. Conclusions

1) Beef products, milk and dairy products, and produce have been associated with outbreaks of non-O157 VTEC (Table 4-6). Non-O157 VTEC have caused illness with the same public health significance as E. coli O157:H7, validated for example, by the outbreak associated with consumption of fenugreek sprouts contaminated with E. coli O104:H4 in Europe in 2011 [4]. However, in general, many non-O157 VTEC do not have the capacity to cause HUS, large outbreaks, or even human infection. This presents a significant dilemma for practitioners of clinical medicine and public health to identify high-risk VTEC at the interfaces between humans, animals, food products and the environment [33].

2) The non-O157 VTEC serogroups implicated in human infections differ between countries. In Canada, clinical samples are not routinely screened for non-O157 VTEC serogroups. Data from the NML’s reference service activities show the six most commonly reported O groups associated with serious illness between 1998 and 2012 to be O26, O103, O111, O117, O121, and O145 (NML, unpublished data). These results were generated from isolates of non-O157 E. coli with confirmed VT producing status.

3) There are no published studies that estimate the dose-response for non-O157 VTEC as a group of pathogens. The development of a single dose-response relationship for non-O157 VTEC is limited by the diversity of clinical outcomes reported for this group of pathogens. There are more than 200 serotypes reported for this group of pathogens, thus, choosing one or a few of these to represent the group may not result in an accurate prediction of risk when applying the dose-response in a quantitative risk assessment.

4) Outside of the VT determinants which define the non-O157 serogroup, the precise contribution of all mobile genetic elements to non-O157 VTEC virulence is unknown [33]. There is a considerable diversity in the range and types of virulence factors associated with non-O157 VTEC infections in humans, which makes defining single traits of virulence difficult [12]. As discussed herein, other virulence genes, such as attachment and colonization factors, as well as other cytotoxins, are needed for pathogenesis to occur. Thus, detection of VT alone or of the genes encoding these verotoxins, in a given isolate, does not provide enough information to classify the health concern of that isolate in the absence of epidemiological information.

5) There are a number of published methods for the detection and isolation of non-O157 VTEC in foods and their validation and or implementation in various settings is in progress. The efficacy of the methods developed by the Canadian Federal VTEC Working Group in the recovery of four tested non-O157 serogroups as well as O157:H7 from ground beef was demonstrated [140, 158]. Furthermore, the method has been expanded to include the isolation and identification of the USDA-FSIS six most common non-O157 VTEC serogroups [143-144]. The USDA also has developed a method to support the implementation of a testing program for the 6 serogroups in food samples [145].

6) Various food processing interventions in the commodities investigated in this risk profile have been shown to control equally well, both E. coli O157:H7 and non-O157 VTEC.

9. Acknowledgements

The authors would like to acknowledge the contributions of the following persons during the preparation of this document: Dr. Matthew Gilmour, Dr. Celine Nadon, Dr. Kirsten Mattison.
10. References


