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# **Review of the State of Knowledge on Verotoxigenic *Escherichia coli* and the Role of Whole Genome Sequencing as an Emerging Technology Supporting Regulatory Food Safety in Canada**

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## **Preface**

The Deputy Heads of the Canadian federal food safety partner agencies (Public Health Agency of Canada, Health Canada and Canadian Food Inspection Agency) have identified the application of emerging science to support the management of food safety issues as a key priority for the modernization of regulatory food safety. One example of an emerging science is whole genome sequencing (WGS), a technology that provides detailed characterization of the genetic content of organisms, such as foodborne pathogenic bacteria. WGS supplements traditional approaches based on phenotypic properties and has the potential to provide more information than is currently being exploited. There is potential to mine genomic data, to go beyond established uses for surveillance and outbreak response in order to inform future food safety decision making, policy and program development, and early interventions to respond to or prevent illnesses and outbreaks. The application of WGS technology in the analysis of verotoxigenic *Escherichia coli* (VTEC) was deemed a good fit because of the importance of this pathogen to public health and the need for clear guidance in determining which characteristics of foodborne VTEC must be considered in identifying isolates most likely to cause serious human illness. This treatise examines the current state of knowledge with respect to the salient characteristics of VTEC of public health concern, the relevant activities of the federal health portfolio food safety partners, and how WGS technology might best be leveraged in identifying the key genomic markers of foodborne isolates to support regulatory food safety objectives

## **Executive Summary**

Verotoxigenic *Escherichia coli* (VTEC) are recognized as a significant foodborne hazard, and consequently, have been the subject of regulatory food safety inspection programs aimed at reducing the burden of foodborne illness in Canada. Current food testing protocols use a decade's old definition of VTEC of public health significance predicated on inclusion of a small number of O serogroups, carriage of verotoxin types 1 and 2, and the *eae* gene coding for intimin, a protein involved in host colonisation. Recent worldwide food safety trends and public health data indicate that there is a need to revise this outdated definition to include other VTEC, which have emerged as food safety hazards. The Emerging Science Whole Genome Sequencing Working group reporting to the Federal Committees on Food Safety has undertaken an extensive review of Canadian public health data and the scientific literature to investigate whether a better definition of VTEC of public health significance can be achieved in order to meet contemporary public health trends. To support understanding of the context in which genomic data is applied to food safety decision making this review also addresses the topics of the foods associated with VTEC exposure, existing monitoring programs for VTEC illness and food contamination, analytical methods for VTEC and risk mitigation efforts in Canada.

It was determined that VTEC are a complex family of pathogens with significant variability in the presence of specific virulence factors, and that the scope of analysis and decision-making should not be limited to specific serogroups, since Canadian public health data show that infections with diverse serotypes may occur. Next generation sequencing technologies are well suited for the analysis of food and clinical isolates of VTEC to identify genetic characteristics, which can inform hazard characterisation. A number of knowledge gaps have been identified which if addressed through focused research have the potential to improve understanding of the factors determining VTEC virulence.

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## **1. Introduction**

Verotoxigenic *Escherichia coli* (VTEC) are a significant cause of bacterial foodborne illness in Canada and worldwide. There are a number of different types of pathogenic *E. coli* (pathotypes), but VTEC are the only *E. coli* pathotype that is nationally notifiable in Canada. The public health significance of VTEC is due to the high infectivity of some strains, the potential for life-threatening illness and the limited treatment options available. However, it is recognised that differences in the virulence potential among VTEC strains exist, and the clinical outcomes reported for this group of pathogens ranges from severe to mild.

In Canada, Whole Genome Sequencing (WGS) is becoming an important method for the characterisation of bacterial pathogens, including VTEC, by those authorities concerned with public health and food safety. Genomic characterisation of bacterial isolates by WGS offers a number of advantages over the established mix of molecular and phenotypic methods used for isolate characterisation, notably once the wet laboratory chemistry work of isolate sequencing has been completed, diverse genomic traits can be assessed through computational analysis. This offers significant savings to the cost and time of isolate characterisation. Additionally, subtyping utilising WGS data can potentially offer a high level of discrimination. Consequently, the expanded use of WGS data analysis offers opportunities to improve human foodborne disease surveillance, outbreak response, health risk assessment and regulatory food sampling.

The purpose of this document is to assist federal health portfolio food safety partners (FFSP) with the utilisation of genomic data for the characterisation of VTEC and to support those researchers seeking to develop and apply genomic characterisation of VTEC for the purposes of food safety. To this end, this document includes a summary of established information regarding VTEC as foodborne pathogens, presented within the context of the regulatory and data collection activities of the FFSP. By providing both summary and context this document will assist FFSP in identifying knowledge gaps which might be addressed through further research and improved data collection practices.

## **2. Mandates and Roles of Canadian Federal Health Portfolio Food Safety Partners**

### **2.1. Canadian Food Inspection Agency**

The Canadian Food Inspection Agency (CFIA) is a science-based regulatory body dedicated to protecting the health and well-being of Canadians by safeguarding Canada's food supply and the plants and animals upon which safe, high-quality food depends. It is the body responsible for administering and enforcing federal statutes and regulations which regulate the safety and quality of food sold in Canada, while supporting a sustainable animal and plant resource base. The CFIA shares many of its core responsibilities with other federal departments and agencies, with provincial, territorial and municipal authorities, private industry, and national/international stakeholders.

The CFIA works with its partners to: implement food safety measures; manage food, animal and plant risks, incidents and emergencies; and promote the development of food safety and disease control systems to maintain the safety of Canada's high-quality agriculture, agri-food, aquaculture and fishery products. The Agency's activities include: verifying the compliance of domestic and imported products; registering and inspecting establishments; testing food, animals, plants and their related products; approving the use of many agricultural inputs; and, research and development to support monitoring programs and food safety investigations

With respect to food safety, CFIA is responsible for assessing the compliance of imported and domestic food with policies, regulations, standards and guidelines set by Health Canada. Where potential issues

with compliance are identified CFIA and/or Health Canada performs a risk assessment to determine the level of risk posed to Canadian consumers. Each food safety incident is assessed, taking into consideration numerous factors such as volume of distribution, hazard type, processing and consumer handling practices, while balancing the weight of evidence and applying appropriate precautions. Risk assessments are one of the inputs used by the Agency when making risk management decisions.

## **2.2. Health Canada**

Health Canada is the federal department responsible for helping Canadians maintain and improve their health. As a science based department and working with various partners Health Canada works to prevent and reduce risk to individual health and the overall environment; promote healthier lifestyles; ensure high quality health services that are efficient and accessible; integrate renewal of the health system with longer term plans in the areas of prevention, health promotion and protection; reduce health inequalities in Canadian society; and provide health information to help Canadians make informed decisions.

In its role as a regulator, Health Canada is responsible for establishing policies, regulations, standards and guidelines related to the safety and nutritional quality of all food sold in Canada.

The Food Directorate provides health risk assessments (HRA) on food-related hazards to the CFIA or other stakeholders (e.g., Provincial/Territorial governments) as well as scientific advice and analytical surge capacity for analyzing microbiological contaminants, chemical contaminants, non-permitted food additives, chemicals associated with the use of food packaging materials, processing aids, and incidental additives, and undeclared food allergens in food and clinical samples.

The Food Directorate also conducts scientific assessments of risk, benefit, and efficacy in support of standard-setting and food safety investigations. It provides information and authoritative advice to the public, industry, health and consumer organizations, and other government organizations. The Food Directorate also conducts scientific research and post-market surveillance to support its standard-setting, risk assessment, and informational roles.

## **2.3. Public Health Agency of Canada**

In Canada, within the provincial and territorial jurisdictions, provision of public health services lies primarily at the municipal or local level. Provincial and territorial governments provide support for local public health services with assistance in the planning, administration of budgets, and the provision of technical assistance. While public health is primarily a provincial/territorial priority, the federal government has authority to legislate aspects of public health as set out in the Department of Health Act and the Public Health Agency of Canada Act.

The Public Health Agency of Canada (PHAC) was established in September 2004, following recommendations in response to the 2003 outbreak of Severe Acute Respiratory Syndrome (SARS). The main mandates of PHAC are to contribute to federal efforts to identify and reduce public health risk factors and to support national readiness for public health threats, including carrying out disease surveillance and control activities, with an emphasis on promoting cooperation with provincial and territorial governments.

PHAC facilitates interactions with provincial and territorial governments, and other stakeholders, and coordinates enteric disease surveillance across the country. Disease surveillance conducted by PHAC has six main objectives:

1. Timely identification of outbreaks and monitoring of trends and antimicrobial resistance of foodborne, waterborne and environmental infectious diseases;
2. Detection of increases among vectors that indicate potential increased risk of infection;
3. Identification of risks to inform risk management, policy and regulation development;
4. Provide evidence for the development, monitoring and assessment of interventions and food safety programs for reducing the burden of disease and anti-microbial resistance;
5. Provide timely public health data on new and emerging disease strains to support the development of required diagnostic tests;
6. Contribute to international surveillance efforts and meet international commitments, including the World Health Organization (WHO) International Health Regulations.

Surveillance includes the monitoring of enteric diseases, most of which are notifiable, and for which there are global reporting commitments to the WHO. These surveillance programs provide data on disease trends, incidence, outbreak investigations, characteristics (in terms of person, place and time), burden of diseases (symptoms, hospitalization, death, length of illness), and risk factors (travel, food, water, and animal exposures), including monitoring the occurrence of pathogens and resistance in exposure sources (animals, food, environment) and exposure factors such as antimicrobial use in animals.

Due to the complexity of enteric diseases, PHAC has established more than one surveillance system to conduct comprehensive, national surveillance (see Section 6 for more details on these systems). Depending on the surveillance system, the information provided to PHAC could be laboratory based (e.g. case counts per disease identified in a week) or public health based (i.e. consisting of case-level information). All of the data collected through these systems are summarized and presented in annual reports, to provide data to support regulators conducting risk assessments, to improve understanding of emerging or existing sources of infection, inform food safety investigations (and potentially food recalls), and contribute to the identification of the most important sources and pathways of each enteric pathogen that cause illness in Canada.

### **3. Verotoxigenic *Escherichia coli***

#### **3.1. VTEC: Definition**

Verotoxigenic *Escherichia coli* (VTEC) are members of the bacterial species *E. coli* with the potential to express one or more verotoxins as determined by detection of the verotoxin protein or possession of the verotoxin gene (*stx*). Verotoxins are bacterial protein toxins of the AB5 family, homologues of the Shiga toxin of *Shigella dysenteriae*, which terminate protein synthesis in cells by cleavage of a specific adenosine residue from the 28S RNA of the 60S ribosomal subunit (Melton-Celsa, 2014). Verotoxin is also known by the synonymous terms verocytotoxin, Shiga-like toxin and Shiga toxin. These synonyms arose because verotoxin was first identified and reported (Konowalchuk et al., 1977) prior to the establishment of its relationship to Shiga toxin (O'Brien and La Veck, 1983). Thus, the terms verotoxin-producing *E. coli*, Shigatoxigenic *E. coli* and Shiga toxin-producing *E. coli*, should be recognised as synonyms for this class of pathogens.

Subgroups of VTEC with additional biological markers or epidemiological association with severe patient outcomes have been identified in the literature with the terms Enterohemorrhagic *E. coli* (EHEC) or



Hemolytic Uremic Syndrome associated *E. coli* (HUSEC). The term VTEC is used throughout this document as it is inclusive of these *E. coli* pathogens.

In addition to VTEC, there are several other enteric pathotypes of *E. coli* which are distinguished on the basis of specific virulence factors, disease symptoms and pathology (Croxen et al., 2013). In addition to *stx*, VTEC strains may possess virulence factors or other biomarkers associated with these pathotypes.

### 3.2. VTEC as a Health Hazard

Following ingestion, VTEC can cause enteric illness with a range of symptoms. In the mildest form of the disease, patients develop self-resolving, uncomplicated diarrhoea. Alternatively, patients may develop bloody diarrhoea (BD) or haemorrhagic colitis (HC), which is not always, distinguished clinically, but in both cases the patient experiences blood in the stool (Karpman and Ståhl, 2014). Consequently, this document will refer to BD when discussing both BD and HC. Though the majority of BD cases will self-resolve, a minority of cases will progress to Haemolytic Uremic Syndrome (HUS). The rate of HUS development varies between outbreaks of both VTEC O157 and other VTEC, with reported rates of HUS development of 2 to 22% (Vallis et al., 2018). HUS is a life-threatening illness resulting in death or end stage renal disease in 12% of cases, and with 25 to 30% of survivors experiencing long term renal sequela (Garg et al., 2003; Garg et al., 2009; Spinale et al., 2013).

Determining the relative hazard posed by VTEC isolates from food remains a major challenge as individual strains appear to vary significantly in the likelihood of causing severe illness, with outcomes such as BD, HUS and death. Some clonally related groups of VTEC, such as VTEC serotype O157:H7, have a higher association with severe patient outcomes and outbreaks than others. Other strains have not been reported as clinical isolates, or have only been associated with cases of uncomplicated diarrhoea. Understanding the pathogenic potential of VTEC strains is further complicated by the apparent role of individual patient factors. Even in outbreaks of high risk VTEC, such O157:H7 or sorbitol-fermenting O157, the symptoms experienced by individuals may range from asymptomatic infection to HUS (Jaakkonen et al., 2017; Bayliss et al., 2016). Similarly, a Japanese study of 399 VTEC isolates from asymptomatic healthy adults found that though many of the isolates possessed serotypes and genotypes that are rarely isolated from symptomatic individuals, strains of VTEC with features associated with BD and HUS were also present (Morita-Ishihara et al., 2016).

In the absence of animal models which mimic human responses to VTEC infection, it is challenging to experimentally determine the factors governing the pathogenic potential of VTEC (NACMCF, 2019). Consequently, many putative virulence factors have been identified on the basis of epidemiological association with symptoms of severe illness (BD and HUS) and association with reported outbreaks. Unfortunately, in this approach there is an inherent risk of confounding the three factors which can be expected to determine the apparent pathogenic potential of individual strains:

- a. The probability that the pathogen strain will come into contact with humans. This will be determined by the ecology of the strain.
- b. Infectivity: the probability of infection on exposure to a single cell of the strain.
- c. The probability of infection resulting in severe illness. Patients suffering severe symptoms, such as BD, are more likely to seek medical attention and be reported.

These three factors can potentially occur in any combination. From a food safety perspective, the problem is that an isolate with previously unreported characteristics may have high infectivity and potential to cause BD and HUS, but has previously been unreported due to a low probability of

exposure. This appears to have been the case with the VTEC O104:H4 strain with enteroaggregative virulence factors, responsible for the 2011 European outbreak (Beutin and Martin, 2012).

### **3.3. VTEC Infectivity**

The infectivity of infectious agents is often described in terms of dose response, the probability of a specified response (i.e., illness, infection, or certain sequelae) following exposure to a specified pathogen in a specified population, as a function of the dose (WHO/FAO, 2003).

Dose response is a concept originally developed for characterising toxicological hazards and presumes a proportional relationship between the concentration of an agent and the severity of the resulting illness. However, for infectious agents, the severity of the illness is not determined by the number of cells or virus particles to which an individual is exposed. The severity of illness is instead determined by the virulence factors possessed by the infecting organism and the immunological vulnerability of the infected individual. Thus, the probability of infection can be characterised as either: infectivity, the probability of infection on exposure to single infectious cell or virus particles; or infectious dose, the number of single infectious cell or virus particles which has a high probability of establishing an infection.

Due to the potential for long term sequela or death, and the absence of effective treatment, experimental determination of VTEC infectivity is not possible. Instead, the infectivity or infectious dose of VTEC has been estimated from data on exposure levels in outbreak foods. Published estimates of the infectivity of VTEC are based on outbreaks of VTEC O157:H7.

A commonly quoted value for the infectious dose of VTEC O157:H7 is less than 100 CFU, based on a review of reported exposure levels from eight outbreak reports (Todd et al., 2008). However, the term infectious dose implies that there is an exposure below which there is negligible probability of infection in otherwise healthy adults. This interpretation is misleading for VTEC as the probability of infection may be significant upon exposure to a single cell.

In a model developed from exposure data from eight outbreaks (six foodborne, one water, one mud) of VTEC O157:H7, the probability of infection was estimated at between 1% and 10% (Teunis et al., 2008). Higher infectivity has been estimated in individual outbreaks, the mean probability of infection per cell, in an outbreak involving salad with seafood sauce was 26% for children and 17% for adults (Teunis et al., 2004). The differences in infectivity estimates from individual outbreaks may be due to multiple variables, including differences in the virulence factors possessed by the infecting strain, the protection of ingested cells from digestion processes by the food matrix, and host vulnerability.

The conclusion that there is significant probability of infection with exposure to a single cell of VTEC O157:H7 is supported by the low levels of the pathogen reported in variety of outbreak food vehicles (Strachan et al., 2001; Gill and Oudit, 2015; Hara-Kudo and Takatori, 2011; Gill and Huszczyński, 2016). It is currently unknown how variable infectivity is between individual VTEC strains or wider phylogenetic groups. Comparison of the levels of non-O157 VTEC in outbreak food vehicles, with reports for VTEC O157, indicates that the infectivity of non-O157 VTEC strains can approach that of VTEC O157:H7 (Paton et al., 1996; Buvens et al., 2011; Gill et al., 2019a).

### **3.4. Features of Vulnerable Populations**

It has long been recognised that the young and elderly are at greater risk of VTEC illness and experiencing severe health outcomes. This relationship between age, the risk of illness, and of severe

illness is illustrated by US data summarised in Table 1 (CDC FoodNet Fast, 2018). The rate of infection is highest for children under 5 years at 8.08 per 100,000. The rate declines steady with each increased age cohort to a minimum of 0.80 per 100,000 for 40 to 49 years old. The rate of infection then increases with age to a maximum of 1.48 for those 70 years and older. Similar patterns are seen for the rate of hospitalisation and death due to VTEC. Though patients over 70 years have the greatest risk of death, 0.06 per 100,000, of any age group, including under 5 years, 0.04 per 100,000.

Females have a higher rate of infection (2.19 per 100,000) and hospitalisation (0.66 per 100,000) than males (1.90 per 100,000 and 0.57 per 100,000), but the death rates of the two genders are the same. This gender imbalance of infection could be attributed to differences in food consumption patterns, particularly raw fruits and vegetables, as proposed to explain the predominance of female patients in the 2011 outbreak of a VTEC/Enterohemorrhagic *E. coli* (EAEC) hybrid associated with sprouts (Frank et al., 2011).

Individual serology has also been proposed as a factor governing the likelihood of VTEC illness (Karmali, 2018). The regular occurrence of infected asymptomatic individuals in outbreaks of VTEC strains that can cause BD and HUS is well documented (Jaakkonen et al., 2017; Bayliss et al., 2016; Kanayama et al., 2015). Mohamed Karmali (2018) noted that the presence of antibodies to the Locus of Enterocyte Effacement (LEE) colonisation factor intimin is associated with protective immunity to VTEC illness. Karmali has hypothesised that mid-twentieth century improvements to sanitation in industrialised countries resulted in a "... reduction in EPEC outbreaks in the 1970's and 80's..." leading "... to a decline in population immunity to intimin which, in turn may have contributed to the emergence of VTEC O157:H7 outbreaks and HUS in industrialised countries in the 1980s." If this hypothesis is correct, it can be expected that individuals who have experienced higher levels of exposure to environmental *E. coli*, particularly Enteropathogenic *E. coli* (EPEC), may have lower likelihood of developing illness from LEE positive VTEC strains.

### 3.5 Summary

The primary features of VTEC as a pathogen are summarised below:

- VTEC are *E. coli* with the potential to express one or more verotoxins (Shiga toxins). Synonyms for VTEC are verotoxin-producing *E. coli*, Shigatoxigenic *E. coli* and Shiga toxin producing *E. coli*. The term VTEC is inclusive of Enterohemorrhagic *E. coli* and Hemolytic Uremic Syndrome associated *E. coli*.
- VTEC infection follows ingestion, with potential outcomes including, asymptomatic infection, uncomplicated diarrhoea, bloody diarrhoea, haemolytic uremic syndrome and death.
- Individual health status contributes to the probability of serious illness.
- Strains of both VTEC O157 and non-O157 VTEC can be highly infectious, with a significant risk of infection on exposure to a single cell.
- The rates of VTEC infection, hospitalisation and death from VTEC illness varies with age. Midlife adults have the lowest rates, with higher rates for the young and old. Children under 5 years of age and adults over 70 have the highest death rates.

- Females have a higher rate of infection and hospitalisation than males, but the death rates of the two genders are the same.

#### **4. VTEC Virulence Markers and their Association with Severity of Illness**

Though many potential virulence markers have been identified in VTEC, the quality of the evidence supporting their role can vary greatly. The following short review focuses on virulence markers for which there is well established evidence, and distinguishes between markers identified through a causal or correlative relationship with human illness as follows:

*causal*, the relationship between a virulence marker and illness or the likelihood of severe patient outcomes (BD and HUS) is based on a known mechanism.

*correlative*, an epidemiological relationship between the virulence marker and the likelihood of severe patient outcomes has been established, but a biological mechanism has yet to be identified. The virulence marker may not be involved in the mechanisms of illness. The epidemiological association with severe patient outcomes may represent correlation with one or more unidentified mechanisms.

It is important to identify whether a virulence marker has been identified on the basis of a causal or correlative relationship as this allows appropriate weight to be put on specific pieces of evidence when making decisions, and indicates issues to be resolved by future research.

##### **4.1 Verotoxin**

The genes for verotoxin (*stx* or *vt*) are encoded by a lysogenic phage, the *stx*-phage, which integrates into the bacterial chromosome (Krüger and Lucchesi, 2015). Thus, *stx* genes may be inherited by daughter cells or acquired by infection by a bacterium with *stx*-phages, which permits continual creation of new VTEC strains by horizontal gene transfer. *E. coli* cells can be potentially infected by multiple *stx*-phages, and VTEC strains which carry genes for two or three different verotoxins are not unusual.

VTEC and *Shigella dysenteriae* are established human pathogens for which verotoxin/Shiga toxin is a virulence factor, but there have been reports of isolates of other genera (*Acinetobacter*, *Aeromonas*, *Citrobacter*, *Enterobacter*) which have acquired verotoxin genes (Probert et al., 2014; Grotiuz et al., 2006; Alperi and Figueras, 2010; Schmidt et al., 1993; Paton and Paton, 1996). Strains of *Escherichia albertii* positive for the genes for verotoxin variant 2f have also been reported (Ooka et al., 2012). There is currently no consensus on the public health significance of verotoxin positive bacteria other than *E. coli*.

On the basis of amino acid sequence and serological reaction, verotoxins can be divided into two main types, VT1 and VT2 (Strockbine et al., 1986). VT2 has a greater epidemiological association with BD and HUS than VT1 (Ostroff et al., 1989; Boerlin et al., 1999). VT2 also displays greater cytotoxicity and is more likely to produce a HUS like pathology in experimental models (Fuller et al., 2011; Donohue-Rolfe et al., 2000; Siegler et al., 2003).

VT1 and VT2 can be further subdivided into subtypes. The toxin subtypes were originally identified on the basis of serological properties but subtypes are now defined by amino acid sequence. Currently, there is an established taxonomy of three VT1 (a,c,d) and seven VT2 (a,b,c,d,e,f,g) subtypes (Scheutz et al., 2012). Since 2012, at least five additional VT subtypes have been proposed: VT1e (Probert et al.,

2014), VT2h (Bai et al., 2018), VT2i (Lacher et al., 2016), VT2k (Meng et al., 2014) and VT2l (Lacher et al., 2016) (Table 2). Epidemiological evidence indicates that some VT subtypes have a higher correlation with severe disease than others (Friedrich et al., 2002; Persson et al., 2007; Buvens et al., 2012; Marejková et al., 2013; Mellmann et al., 2008). Epidemiological data indicates that two subtypes, VT2a and VT2d, are associated with a greater likelihood of BD and HUS, and two subtypes, VT1a and VT2c, are associated with an increased likelihood of BD (FAO/WHO, 2019; NACMCF, 2019). The association of *stx*-subtypes with severe disease also involve correlation with the presence of other virulence factors, particularly those involved in colonisation (FAO/WHO, 2019; NACMCF, 2019). The remaining VT subtypes may have a greater correlation with mild disease and lower association with BD and HUS, but VTEC strains with these VT subtypes cannot be excluded as potential pathogens and as a cause of serious illness. Though rare and typically involving individuals with a vulnerable health status, there have been reports of BD and HUS involving VTEC carrying VT1c (Lienemann et al., 2012), VT2b (Stritt et al., 2013), VT2e (Fasel et al., 2014; Thomas et al., 1994) and VT2f (Friesema et al., 2014; Friesema et al., 2015; Grande et al., 2016). VTEC strains carrying VT1d and VT2g have been isolated from cases of relatively mild enteric illness, but not from patients with BD or HUS (Nüesch-Inderbinen et al., 2018; Scheutz 2014; Prager et al., 2011). The significance of the 5 additional subtypes proposed since 2012 is unclear, VT2h, VT2i, VT2k, and have only been reported as isolates from animals, and though VT1e was reported in a clinical isolate, it was a strain of *Enterobacter cloacae* (Probert, et al., 2014; Bai et al., 2018; Lacher et al., 2016; Meng et al., 2014).

Caution should be taken in interpreting studies correlating severe disease with specific verotoxin subtypes as there are two important potential sources of bias. Firstly, studies conducted prior to the establishment of the current subtype taxonomy may have categorized subtypes differently. Secondly, molecular and antibody-based screening methods for VTEC may not detect all verotoxin subtypes (Feng et al., 2011; Staples et al., 2017; De Rauw et al., 2016). Additionally, the VTEC isolated from a patient may not necessarily be the causal agent of the symptoms observed. Coinfection of a patient suffering from HUS with two strains of VTEC which possessed different serotypes and virulence gene profiles has been reported (Gilmour et al., 2007a).

Differences in cytotoxicity may explain the apparent relationship between the VT subtype and the likelihood of severe disease. A study with purified toxins found that VT2a and VT2d were 25 times more toxic than VT2b and VT2c to Vero monkey kidney cells and human renal proximal tubule epithelial cells and 40-400 times more toxic to mice than VT2b, VT2c and VT1 (Fuller et al., 2011). However, factors other than cytotoxicity may play an important role. VTEC may carry multiple copies of VT genes (Ashton et al., 2015), strains carrying the same VT-subtype may differ in their levels of toxin expression (Kimmitt et al., 2000) and, as recently reported, in the rate of translocation across the intestinal epithelium (Tran et al., 2018). A Dutch study of clinical VTEC isolates reported that the presence of specific VT subtypes was correlated positively and negatively with other virulence associated genes (Franz et al., 2015). The authors proposed that the association of VT-subtype with illness may be a consequence not of the properties of the toxin, but of the assemblage of virulence genes associated with that VT subtype among certain VTEC lineages (Franz et al., 2015). This hypothesis does not contradict previous observations of an association with severe disease and VTEC with VT2a and LEE, and strains LEE negative strains with VT2d (Friedrich et al., 2002).

Finally, VTEC carrying some VT subtypes appear to be more common in some environments than others. VT2e is associated with VTEC isolates from pigs, in which they cause edema disease (Tseng et al., 2014). VT2b and VT2c are associated with deer and other wildlife (Hofer et al., 2012; Mora et al., 2012). A study of VT2f positive VTEC isolated from pigeons or human sources in Holland reported that they clustered

phylogenetically by isolation source with limited overlap (van Hoek et al., 2019). The VT2h subtype has only been reported in VTEC isolated from Tibetan marmots (Bai et al., 2018). Thus, the probability of human exposure to different verotoxin subtypes may contribute to the epidemiological relationship with disease.

#### **4.2 Locus of Enterocyte Effacement (LEE)**

The LEE pathogenicity island is a complex suite of genes that function together to regulate colonisation of the intestinal epithelium by the formation of attaching-effacing (A/E) lesions (Stevens and Frankle, 2014). The LEE is encoded upon a mobile genetic element; its presence is typically determined by detection of the presence of the gene *eae*, which encodes intimin, a protein that is essential for bacterial attachment to gut mucosal epithelial cells, thus serving as an essential element in the establishment of infection. The LEE is the definitive virulence factor of EPEC, but may also be present in VTEC strains (Croxen et al., 2013). EPEC cause acute self-limiting diarrhoea and are estimated to be much less infectious than VTEC, with infection in volunteer exposure studies requiring ingestion of >1 million cells (Todd et al., 2008).

The capacity of *E. coli* O157:H7 to create A/E lesions was first reported in 1986 (Tzipori et al., 1986) and shortly after Levine (1987) proposed that the capacity to create A/E lesions was diagnostic of *E. coli* O157:H7 and related *E. coli* pathogens. Since then the role of LEE and the mechanism of A/E lesion formation has been identified (Stevens and Frankle, 2014). It is now well established that LEE positive VTEC have a higher association with BD and HUS (Boerlin et al., 1999; Brooks et al., 2005, Ethelberg et al., 2004; Naseer et al., 2017). However, the virulence mechanism of LEE is not simple. Integral to the LEE is an encoded Type Three Secretion System by which the bacterium translocates proteins into host cells (Gaytán et al., 2016). The translocated proteins include a core group of LEE encoded effectors which are essential for A/E lesion formation, such as the translocated intimin receptor (*tir*), as well as other non-LEE encoded effectors. The presence of specific effectors may vary between *E. coli* with LEE (Santos and Finlay, 2015). This variability in the specific effectors present as well as, variability in protein sequence and the translation rates of those effectors present, may contribute to differences in the pathogenicity potential of individual strains.

#### **4.3 Aggregative Adhesion (AA)**

AA of the intestinal epithelium is typical of infectious colonisation by EAEC. EAEC are a cause of acute diarrhoeal illness in healthy adults and may cause persistent diarrhoea in infants and immune compromised adults (Hebbelstrup Jensen et al., 2014). The definitive EAEC virulence factors are Aggregative Adherence Fimbriae (AAF). The AAF is encoded on a plasmid, pAA, along with a suite of proteins with roles in adhesion, biofilm formation, the regulation of plasmid and chromosomal genes, and toxins (Hebbelstrup Jensen et al., 2014; Boisen et al., 2014). The presence of the plasmid can be detected by the presence of the gene *aggR*, which encodes a regulatory protein. Since the plasmid may be lost during cell replication, a chromosomally encoded gene *aaIC* can also be used to identify EAEC (EFSA, 2015).

The potential role of AA in the pathogenicity of HUS associated VTEC was identified in 1998, following molecular analysis of the VTEC O111:H2 strain associated with a 1992 outbreak in France (Morabito et al., 1998). However, AA carrying VTEC was not generally considered a significant pathogen until the European outbreak of VTEC O104:H4 in 2011. Centred in Germany, with over 4000 cases of illness and 53 deaths this outbreak is among the largest outbreaks of VTEC reported (Beutin and Martin, 2012). This strain was VT2a positive and LEE-negative, but was identified as carrying pAA, it is apparently highly infectious and had a very high rate of HUS (22% of reported cases) (Boisen et al., 2015). Genomic

analysis revealed that AA VTEC O104:H4 had originated as an EAEC which had been infected with the *stx*-phage (Rasko et al., 2011). Consequently, such strains are designated VTEC/EAEC or *stx* positive EAEC.

Subsequent to the 2011 outbreak, it has been proposed the VTEC/EAEC hybrid strains be recognised as a class of high risk VTEC strains (WHO/FAO, 2018). To date there has been no further large-scale outbreaks of VTEC/EAEC, but there have been reports of their isolation from clinical cases (Dallman et al., 2012; Prager et al., 2014). Retrospective analysis of Italian clinical isolates revealed that a VTEC/EAEC O104:H4 strain, related to the 2011 outbreak strain, had been isolated in a sporadic case of HUS in 2009 (Scavia et al., 2011). Though a relatively rare subgroup of VTEC, VTEC/EAEC cases have probably gone unrecognised historically due to the failure to test VTEC isolates for the presence of EAEC virulence genes. Thus, there may be VTEC/EAEC strains currently circulating that have yet to be identified and new strains of VTEC/EAEC will likely arise in the future, due the mobility of the primary virulence genes, *stx* and pAA.

As with the LEE, AA colonisation and pathogenicity involves a complex of genes. The genes involved are not encoded solely on the pAA but also chromosomally and individual EAEC strains may possess variants of the genes or some may be absent (Hebbelstrup Jensen et al., 2014). This variability may contribute to differences in the pathogenic potential of individual VTEC/EAEC strains.

#### **4.4 Locus of Adhesion and Autoaggregation (LAA)**

A third suite of virulence genes involved in colonisation of the gastrointestinal tract has recently been identified in LEE-negative VTEC. The LAA is a pathogenicity island that to date has only been reported in strains of LEE-negative VTEC (Montero et al., 2017; Colello et al., 2018; Montero et al., 2019). The complete LAA pathogenicity island is 86 kb, and composed of four modules (Montero et al., 2017). Individual strains of VTEC may carry one or more modules and strains carrying all four LAA modules have an increased association with BD and HUS (Montero et al., 2017). Module IV contains genes that are widely distributed among pathogenic and non-pathogenic *E. coli* and are therefore not suitable for diagnostic tests, but primer sets for the detection of the modules I, II and III have been published (Montero et al., 2017)

#### **4.5 Other Virulence Factors**

In addition to the LEE, AA, and LAA suites of virulence genes which have an identified role in host colonisation in VTEC, there are numerous other putative virulence genes whose role in determining the pathogenicity potential of VTEC is unknown or uncertain. A selection of these putative virulence genes is included in Table 2. These putative virulence factors include adhesins and toxins, and like the established virulence genes, many are encoded within pathogenicity islands and mobile genetic elements. Examples of such pathogenicity islands identified in LEE-negative VTEC are the Locus of Proteolysis Activity (Schmidt et al., 2001), Subtilase-Encoding Pathogenicity Island (Michelacci et al., 2013), Pathogenicity Island I (CL3) (Montero et al., 2019), and the High Pathogenicity Island of *Yersinia pestis* (Karch et al., 1999). The importance of VTEC with gene assemblages that do not include *eae* and *aaiC/aggR* is illustrated by the epidemiological significance in Europe of HUS associated VTEC O91:H21, which is characterised by genes for VT2d, enterohemolysin (*ehxA*) and cytolethal distending toxin (*ctdB*) (Bielaszewska et al., 2009).

There is currently no consensus on whether the presence or absence of any of these putative virulence factors and pathogenicity islands are predictive of an increased likelihood of severe disease. Specific examples which have been proposed as markers of increased likelihood of severe disease include: *toxB*

(adhesin) in LEE-positive VTEC (Tozzoli et al., 2005), and in LEE-negative VTEC, *saa* (STEC agglutinating adhesion), *subAB* (subtilase toxin), *ureC* (Urease-associated protein), *ehxA* and *ctdB* (Paton et al., 2001; Paton et al., 2004; Khaitan et al., 2007; Bielaszewska et al., 2009; Franz et al., 2015). Though the mechanistic roles in disease processes has not been established for these putative markers, analysis of large numbers of VTEC genomes from differing isolations sources or linked to patient outcome data may provide evidence for their association with human illness (Franz et al., 2015; Montero et al., 2019).

#### 4.6 Hybrid Pathotypes

In a 1998 review paper Nataro and Kaper (1998) established a categorisation of *E. coli* causing enteric illness into enteropathotypes based on the associated pathology and virulence factors. The pathotype categories have evolved and currently accepted pathotypes include EPEC, VTEC, EAEC, Enteroinvasive *E. coli* (EIEC), Enterotoxigenic *E. coli* (ETEC), Diffusely Adherent *E. coli* (DAEC), and Adherent Invasive *E. coli* (AIEC) (Croxen et al., 2013). Since many *E. coli* virulence factors are encoded on mobile elements, including the phage encoded verotoxin genes, it should be expected that strains with hybrid sets of virulence factors will exist. LEE-positive VTEC and VTEC/EAEC illustrate the clinical significance of these hybrids, as can *Shigella dysenteriae*, which can be viewed as essentially a host specialised population of VTEC/EIEC hybrids. Additionally, there have been reports of VTEC strains which carry virulence genes associated with ETEC, including heat stable toxins (Michelacci et al., 2018; Leonard et al., 2016; Nyholm et al., 2015; Bai et al., 2019). Finally, there have been VTEC isolates which do not conform to simple hybrid states as would be produced by the infection of a strain of another pathotype with a *stx*-phage, including a VT1-positive clinical isolate with features of AIEC, EAEC and EPEC strains (da Silva Santos et al., 2015) and VTEC O80 an emerging pathogen in Europe possessing virulence genes previously associated with extraintestinal pathogenic *E. coli* (ExPEC) (Cointe et al., 2018). A graphical representation of the relationship between VTEC and other *E. coli* pathotypes is presented in Figure 1.

#### 4.7 Subtyping

VTEC, as with other bacteria, can be subtyped on the basis of phenotypic (i.e., serology, biochemical profile) or genomic methods to establish taxonomic relationships between isolates within a population. An implicit assumption in all subtyping approaches is that the greater the number of phenotypic or genomic traits shared by two isolates, the more recently they shared a common ancestor. Such relationships can be used to identify potential pathogens, though this role has been largely replaced by analysis for specific virulence traits. Subtyping remains a crucial tool for surveillance, outbreak response and source tracking.

The assumption of a relationship based on shared traits may be unreliable if the traits used to determine the relationship are prone to rapid change, such as horizontal gene transfer (HGT) events, mutation, or are phenotypic traits which can be masked by differential gene expression. For example, HGT of O- and H-antigen loci in *E. coli* is common, which impacts the utility of serotyping for assessing evolutionary relationship among strains (Ingle et al., 2016). When interpreting the results of subtyping, or selecting a subtyping methodology, consideration should be given as to whether the traits selected are appropriate for the classification of isolates for the required purpose. This can be an issue with VTEC as many of the virulence markers, including verotoxin, are encoded on mobile genetic elements.

The second issue relevant to the interpretation of subtyping results is the level of resolution. Subtyping can be too discriminatory or not discriminatory enough, excluding relevant or including irrelevant isolates, depending upon the application. Phenotypic methods for subtyping VTEC, including biochemical tests, serotyping, phage typing and multi-locus enzyme electrophoresis, are well established but resource intensive and provide limited discrimination (Fratamico et al., 2016). There is a



diverse variety of genomic methods for VTEC subtyping (i.e., restriction length polymorphism, ribotyping, multi-locus variable tandem repeat analysis (MLVA), pulsed-field gel electrophoresis (PFGE), multi-locus sequence typing (MLST)) of varying discriminatory power, resource requirements and reliability (Fratamico et al., 2016). Following the establishment of PulseNet USA in 1996 and subsequent international networks, PFGE became the established standard for high-resolution subtyping of VTEC, and other bacterial pathogens for outbreak detection (Ribot and Hise, 2016). Currently, PFGE is being supplanted by methods based upon WGS (Nadon et al., 2017).

In principle, methods based on WGS are capable of providing subtyping information at any level of discrimination required. At the highest level of resolution, the entire genome is considered using methods such as whole-genome MLST (wgMLST) based on species-specific pan-genome allele definitions (Nadon et al., 2017). Core-genome MLST (cgMLST) is a high-resolution typing approach based on a subset of conserved genes that can be implemented using standardized allele definitions developed for the species. The wgMLST scheme for *E. coli* used by PulseNet and implemented in BioNumerics currently includes 14837 accessory gene loci and the 2513 core gene loci used in the Enterobase cgMLST scheme (<http://www.applied-maths.com/applications/wgmlst>, accessed 2019-05-09)(Zhou et al., 2019). PulseNet Canada identifies possible outbreak clusters on the basis of allelic differences between strains characterized by wgMLST on the BioNumerics platform (Nadon et al., 2017). For some applications, such as long-term or global epidemiological studies, lower resolution approaches based upon variability within a smaller subset of genes may be useful. Ribosomal MLST (rMLST) uses 53 conserved ribosomal protein genes using a scheme that is universally applicable to all bacteria (Jolley et al., 2012) and identification of rMLST alleles can be used to assess sequence quality (Low et al., 2019). Historic *E. coli* MLST typing schemes include the 7 gene Achtman scheme (Wirth et al., 2006) and the 8 gene Pasteur scheme (Jauregui et al., 2018) which can be easily derived from the WGS data.

Single Nucleotide Variant (SNV) (also referred to as Single Nucleotide polymorphism (SNP)) analyses can also be conducted to assess variability within a set of strains at a similar level of resolution to wgMLST (Petkau et al., 2017; Nadon et al., 2017). Care must be taken in the interpretation of SNV results as only core genome sequences conserved within the set of strains are included in the analysis, and mobile elements such as phage or plasmids that may be missing in one or more strains within a set of closely-related strains would be excluded from the analysis. Furthermore, inclusion of unrelated isolates will reduce the size of the core genome and thus the discriminatory power of the approach. While WGS-based approaches are now routinely used for identification of clusters of related illnesses, standards for analysis and the interpretation of results, including the number of SNVs or allelic differences necessary to distinguish strains, have yet to be firmly established. Biological considerations, such as differences in rates of mutation in among strains of *E. coli*, could confound cluster identification based on WGS data (LeClerc et al., 1996; Grad et al., 2012).

Another consideration for the implementation of WGS-based approaches is the need to maintain interoperability with historic data which can be met by computational (*in silico*) prediction of typing data. MLST profiles can be reliably determined using a number of tools (Zhou et al., 2019; Maiden et al., 2013; Joensen et al., 2014). *E. coli* serotype can also be predicted based on WGS data using tools such as ECTyper (Le et al., 2018), SeroType Finder (Joensen et al., 2015) or from raw reads with the EcOH database (Ingle et al., 2016). In cases where high-quality closed genomes have been produced using long-read sequencing data, PFGE patterns can be predicted (Babenko and Toleman, 2016); however, for public health purposes WGS assemblies are typically produced using short-read sequencing technologies and PFGE patterns are difficult to predict from the fragmented assemblies produced by these methods. VTEC WGS analysis pipelines implemented in Canadian public health organizations include modules for

subtyping at different levels of resolution including wgMLST, SNV analysis, rMLST, MLST, serotype prediction, virulence profiling and AMR prediction.

#### 4.7.1 Serotyping

Due to its historical importance the interpretation of serotype is a topic that needs to be addressed specifically in regards to hazard characterisation of VTEC. Though serotyping is unreliable for hazard characterisation, it remains significant due to the use of serology to define VTEC of legislative or regulatory concern, and the need to maintain compatibility with historical classification schemes.

Serological typing of *E. coli* is based on the scheme originally developed in the 1940's by Kauffman (1947), and subsequently modified (Ørskov et al., 1977), to allow *E. coli* strains to be distinguished by the binding of antibodies to antigenic structures on the cell surface. An *E. coli* serotype is defined in the Kauffman scheme by 3 antigen types, O, H, and K. The O-antigen is the polysaccharide of the lipopolysaccharide of the outer membrane. The H-antigen is the protein flagellin, which comprises the filament of the bacterial flagellum. The K-antigens are diverse acidic capsular polysaccharides (Scheutz and Strockbine, 2005). *E. coli* isolates are often designated only by the O- and H-antigens, and approximately 186 O and 53 H antigens have been identified (Stenutz et al., 2006). Serotyping is relatively time-consuming and expensive due to the need to test multiple serological reactions and cross reactions. Additionally, a significant proportion of strains cannot be serotyped, either because they produce antigens that do not conform to the Kauffman scheme (untypable) or they do not express the lipopolysaccharide O chain (rough) or flagellin (non-motile). For these reasons serological typing is being superseded by molecular subtyping based on PCR (polymerase chain reaction) or sequencing (Gilmour et al., 2007b; Iguchi et al., 2015; Joensen et al., 2015). Molecular subtyping may not be fully analogous to serological typing, especially for O type. The H antigen is a structural protein encoded by a specific gene, but the O-antigen is a polysaccharide which is the output of multiple synthesis genes, and so indirect molecular markers must be used (DebRoy et al., 2016).

Historically, serotyping has been an important tool for the identification of pathogenic *E. coli* because of the limited phenotypic characteristics which allow pathogenic strains to be distinguished from commensal *E. coli*. The identification of virulence factors and methods for their rapid detection has made serotyping redundant for pathogen identification. Moreover, horizontal transfer of O- and H- loci highlights potential issues with reliability of the approach for assessing strain similarity (Ingle et al., 2016). However, serotype has continued to be used as a factor in the identification of VTEC of public health significance, based on association with outbreaks or severe illness. This type of analysis has been developed into formal schemata, such as the seropathotype classification proposed by Karmali et al. (2003).

*E. coli* O157:H7 and nonmotile (NM) is the most common serotype of pathogenic *E. coli* reported in Canada, constituting 87.6% of 20,926 clinical isolates from 1999 to 2016 (Supplement 1). The serotype of clinical isolates of non-O157 VTEC is diverse, with 71 O-types among confirmed VTEC isolates reported to the PHAC National Microbiology Laboratory between 1998 and 2012 (Table 3). Among 498 non-O157 VTEC isolates the most common serotypes were O26 (14.1%), O121 (12.4%), O103 (11.0%), rough or untypable (10.8%), O111 (8.8%), and O145 (3.2%). The proportion of reported *E. coli* isolates of the O157:H7/NM serotypes has been declining consistently since 2009, in 2015 and 2016 they accounted for only around 70% of isolates (Figure 2). The decline in VTEC O157 as a proportion of reported VTEC could be a consequence of changes within the beef processing industry (Pollari et al., 2017), the VTEC strains that Canadians are exposed to from other sources and changes in clinical testing methods.

In the US, as in Canada, VTEC serotypes O157:H7/NM are the most commonly reported, accounting for approximately 50% to 36% of cases annually reported in the period of 2010 to 2015 (CDC FoodNet, 2017). As in Canada, there has been a decline in the proportion of O157:H7/NM reported in recent years. In the US a group of 7 VTEC serogroups (O157, O26, O45, O103, O111, O121, O145) are considered a public health priority and have been identified legislatively as adulterants of raw ground beef and its precursor material (Gould et al., 2013; USDA-FSIS, 2012). It should be noted that the National Enteric Surveillance Program data indicates an overlap with this group, though serogroup O45 does not fall within the top ten most commonly reported serogroups in Canada (Table 3).

As a low discrimination method of subtyping, serotype indicates a potential clonal relationship between isolates. As such the presence of a VTEC serotype associated with outbreaks or severe illness can be viewed as a marker for the potential presence of currently unidentified virulence, infectivity or ecological factors which can result in increased likelihood of illness. Unfortunately, such an interpretation is not reliable, as the O- and H-antigens are not virulence factors, nor are they known to be genetically linked to any factors that promote pathogenicity in VTEC. Additionally, it should be recognised that the presence of a serotype not previously associated with VTEC causing BD and HUS provides no assurance that the strain cannot cause life threatening illness, as demonstrated dramatically by VTEC/EAEC O104:H4 (Beutin and Martin, 2012).

#### 4.8 Summary

As the preceding review indicates, an extensive set of markers related to the potential pathogenicity of VTEC strains have been proposed. Interpretation of the relative relevance of these markers is complex due to knowledge gaps and the limitations of experimental models. The following summary identifies the primary points of agreement which form the basis for the current scientific consensus (FAO/WHO STEC Expert Group, 2019; NACMCF, 2019).

- VTEC are *E. coli* with the potential to produce verotoxin. *E. coli* strains which do not possess the verotoxin gene, *stx*, are not VTEC; even if they possess the serotype or accessory virulence factors associated with VTEC capable of causing severe illness, such as BD and HUS.
- There are 10 established subtypes of verotoxin. Subtypes VT2a and VT2d have a greater epidemiological association with BD and HUS. VT1a and VT2c are associated with an increased likelihood of BD.
- An increased likelihood of BD and HUS is indicated by the presence of virulence factors involved in adherence of VTEC cells to the epithelium of the gut. Virulence factors with an established role in gut colonisation include:  
The Locus of Enterocyte Effacement (LEE), gene *eae*.  
Aggregative Adhesion (AA), genes *aggR* and/or *aaiC*
- A third set of virulence factors involved in VTEC colonisation of the gut, The Locus of Adhesion and Autoaggregation (LAA) has recently been identified.
- A potential clonal relationship, as indicated by subtyping, between a VTEC isolate and previously reported VTEC strains isolated from cases of BD or HUS can be used as an indicator of increased likelihood of severe illness. Serotyping provides low discrimination subtyping and is not reliable for hazard characterisation of VTEC in the absence of information on the virulence gene profile.

- Numerous additional gene markers have been proposed as indicators of increased pathogenic potential for VTEC. Due to the limitations of experimental models of VTEC disease process, the relationship between gene markers and the likelihood of severe illness is dependent upon the ability to link genomic data from clinical isolates to metadata on patient outcomes.

## **5. Food and VTEC Exposure**

There are very few food types that have not been reported as sources of VTEC illness, including both foods of animal and plant origin. The diversity of foods reported as vehicles of infection in foodborne VTEC outbreaks is presented in Table 4. However, though the range of foods reported as vehicles of VTEC are diverse, a much smaller range of foods can be identified which account for the majority of outbreaks.

### **5.1. Foods Associated with VTEC illness**

The following discussion is based on analysis of 733 incidents of foodborne VTEC, with an identified food vehicle, reported in Canada (n=189) and internationally (United States n=392, United Kingdom n=63, Japan n=18, France n=10, Sweden n=9, etc.) from 1982 to 2018 (Supplement 2). A summary of the data set divided by food type is presented in Table 5. This data set is heavily weighted to North America, with 79.3% of reports from the United States or Canada. This is a result of the two largest national summaries of reports available being the Centers for Disease Control [National Outbreak reporting System](#), which has data from 1998 onwards, and the series Foodborne and Waterborne Disease in Canada (Health and Welfare Canada), which provides reports for foodborne VTEC from 1982 to 1995. VTEC O157 were involved in 86.3% of foodborne VTEC incidents identified worldwide, and 13.7% involved other VTEC serotypes or serotype was not specified (Supplement 2). This dominance of VTEC O157 may partially reflect bias in laboratory and investigatory methods, as foodborne incidents of non-O157 have been reported with increasing frequency since the beginning of the 21<sup>st</sup> century (Figure 3).

Internationally, foods of animal origin accounted for the largest proportion of incidents (65.5%), followed by foods of plant origin (18.7%) and complex foods (where no specific ingredient was identified) 15.8%. The ingredients most commonly identified as vehicles for VTEC were beef (40.5%), raw milk dairy products (9.8%), leafy greens (9.7%) and unspecified meats (5.9%). In Canada, the ingredients most commonly identified as vehicles for VTEC were beef (62.4%), raw milk dairy products (8.5%), unspecified meats (6.3%) and pork (4.8%). However, when considered in terms of the number of cases of foodborne illness, foods of plant origin appear considerably more significant. Internationally, 57.5% of cases of foodborne VTEC illness were associated with foods of plant origin, and in Canada 22.0%. Internationally, the greatest number of illness was associated with sprouts (40.4%), with leafy greens (8.7%), fruits and berries (5.0%), and vegetables (2.6%). In Canada, leafy greens (7.1%), vegetables (7.8%), and fruits and berries (5.5%) have contributed a disproportionate number of cases of illness per outbreak.

The association of specific foods with VTEC illness can be understood with reference to the ecology of the pathogen. *E. coli*, including VTEC, achieves maximal replication rates in a warm, wet, nutrient rich environment, as exemplified by the gastrointestinal tract of mammalian or avian hosts. However, VTEC can colonise a wide range of animal hosts and can persist for prolonged periods under environmental conditions inhibitory to their replication; as such, VTEC and other *E. coli*, are ubiquitous environmental organisms (Jang et al., 2017; Persad and LeJeune 2014). Consequently, there is a sporadic occurrence of

outbreaks associated with foods, which are unlikely to be contaminated with VTEC and are not conducive to VTEC replication, such as flour (Crowe et al., 2017) or nuts (US CDC, 2011; Davidson et al., 2015).

The high association of VTEC exposure with food of animal origin, particularly beef and dairy, arises from the potential for animals to serve as hosts for VTEC (Ekong et al., 2015; Farrokh et al., 2013). This creates a significantly greater likelihood of initial contamination during harvesting (i.e., slaughter and milking). The relative association of VTEC with different meat animals, based on outbreak data, will be determined by the potential for specific host species to be colonised by VTEC strains which cause BD and HUS, as these are more likely to be reported, for example carriage of these types of strains appears more common in cattle than in swine (Ercoli et al., 2015). However, recent outbreaks of VTEC O157 in the province of Alberta linked to pork products indicate this may be an emerging area of concern (Honish et al., 2017; Alberta Health Services, 2018).

Historical reported rates of VTEC prevalence on raw meats in Canada are significantly higher than contemporary rates. A study published in 1990, reported VTEC frequency in ground beef of 36.4% (25 g n=225) and 10.6% in ground pork (25 g n=235) (Read et al., 1990). A study published a decade later reported isolation of VTEC from 30% of raw boneless beef samples (25 g n=120) (Atalla et al., 2000). While more recently, FoodNet testing of retail ground beef from 2014-2017 reported VTEC in 2% of 1,458 samples (25 g), and 6.1% (25 g n=98) in ground pork (Table 6). Similarly, over the last two decades the frequency of VTEC in raw ground beef precursor material has fallen significantly from 30% in 2000 to 1.82% (325-350 g) in 2012, a trend correlated with significant changes in hygiene and decontamination practices at Canadian beef slaughter plants (Pollari et al., 2017).

The frequency of dairy contamination with VTEC is significantly higher than beef, with a 2014 US study finding verotoxin genes in 13.1% of 100 ml samples of bulk raw cow milk (Sonnier et al., 2018). The origin of these pathogens as with other milk microbiota is the surface of the udder (Oliver et al., 2005). However, the probability of VTEC contamination at milking is offset by routine thermal processing, which prevents consumer exposure. Of 97 incidents of foodborne VTEC involving dairy products identified internationally, 72 (74%) involved dairy products specifically identified as raw milk products. In Canada, 89% of incidents involving dairy products identified raw milk products as the exposure vehicle (Table 5). From this, it can be presumed that without routine pasteurisation the association of dairy with VTEC outbreaks would be much higher.

In comparison, surveys of VTEC frequency on leafy greens indicate that it is much less likely to be contaminated with these pathogens. A US survey from 2009 to 2015 reported that the prevalence of VTEC O157 and non-O157 VTEC in 14,183 samples of leafy greens (iceberg lettuce, romaine lettuce, spinach) was 0.01% and 0.07%, respectively (Zhang et al., 2018). CFIA testing of fresh and fresh-cut ready to eat (RTE) fruits and vegetables (n=37,718) did not identify VTEC O157 in any surveys from 2013 to 2018 (Table 7). Thus, the association of VTEC with these products does not reflect a high probability of contamination, instead the popularity of these products, and practice of consuming them raw are probably the major factors making these foods a prominent vehicle of VTEC illness.

From these observations it can be concluded that foods derived from ruminants, such as beef and dairy, will continue to have a relatively high likelihood of VTEC contamination at harvest, but the likelihood of illness can be moderated by decontamination prior to consumption. Since VTEC contamination of leafy greens and, fruits and berries is dependent on relatively rare pre-harvest contamination from diverse origins, opportunities to prevent pre-harvest contamination may be limited and costly. It should be

expected that, without the introduction of effective and routine decontamination treatment prior to consumption, these products will remain a significant source of VTEC illness.

## **5.2. Food Preparation Practices Associated with VTEC Illness**

As discussed, in the previous section, foods of animal and plant origin may both become contaminated with VTEC. Foods of animal origin, particularly from cattle and other ruminants, have a higher probability of being contaminated with VTEC, as the animals may be hosts, or raised or transported with hosts. The probability of contamination of foods of plant origin is much lower, but as noted in section 5.1 outbreaks can involve a disproportionate number of illnesses.

The key risk factor related to food preparation in all food types is consumption of raw or undercooked food. In the case of foods which are typically heat treated, such as fresh meats (cooking) or dairy (pasteurisation), investigations have commonly found evidence of the consumption of undercooked, raw, or ready to eat products (Beutin and Martin, 2012; Cowden et al., 2001; Michino et al., 1999). Therefore, it is important for consumers to be aware of how their food is manufactured and the associated risks, for example raw milk cheeses and needle tenderized meats. Similarly, illnesses associated with contaminated flour were linked to consumption of uncooked dough (Morton et al., 2017). The importance of the consumption of raw or RTE products to the scale of outbreaks is illustrated by the dominance of such food vehicles in the largest foodborne VTEC outbreaks reported internationally (Table 8) and in Canada (Table 9). Therefore, it is important that food be prepared in a hygienic manner to prevent contamination and be cooked according to the recommended guidelines ([Safe Cooking Temperatures](#)).

It is commonly recommended that fresh fruits, vegetables and leafy greens, whether whole, fresh cut or prepackaged, should always be washed prior to preparation and consumption (CDC, 2018). However, although washing may remove visible soil, reducing the level of contamination, it is not a process that can ensure safety.

## **5.3. Levels of VTEC in Outbreak-Associated Foods**

Quantification of VTEC in outbreak food vehicles is not regularly reported and what data is available is primarily for VTEC O157. A table summarising twelve reports of VTEC contamination levels in outbreak associated foods is provided (Table 10). Reported levels range from tens of CFU per g to below 1 MPN per 100 g. What is apparent from this data is that outbreaks can result from foods contaminated with VTEC at levels below 1 cell per 25 g. As 25 g is the analytical unit most commonly recommended for foods other than raw ground beef (RGB) and RGB precursor, it is clear that robust sampling plans are required to ensure detection of VTEC in foods at levels that can potentially cause outbreaks.

## **5.4 Summary**

The following features describe the relationship between food types and outbreaks of VTEC illness:

- The range of foods implicated in outbreaks of VTEC illness are very diverse including, meats, dairy, vegetables, fruits, nuts, seafood, wheat flour etc.
- Based on the number of reports of foodborne outbreaks of VTEC illness related to specific food types, the most common sources of VTEC exposure are meat (particularly beef), dairy (particularly raw milk products) and leafy greens.

- Though less likely to be a cause of outbreaks than foods of animal origin, more cases of illness are associated with foods of plant origin.
- Consumption of raw or ready-to-eat foods increases the probability of VTEC illness.
- The levels of VTEC in outbreak associated foods are variable, with contamination levels ranging from tens of CFU per g to below 1 MPN per 100 g.

## **6. VTEC Illness Monitoring In Canada**

Surveillance for foodborne pathogens is critical to the planning and management of food safety programs as it aims to detect and prevent foodborne infections, while providing evidence that the food moving through the food chain is safe for consumers. Surveillance systems typically collect information from multiple sources to provide a picture of a particular disease in the community, potential sources and to identify areas where control points can be introduced. As a result, a foodborne surveillance system aims to:

- Determine the magnitude and monitor trends of foodborne infections;
- Identify outbreaks of foodborne infections at an early stage to implement control measures, including product recalls;
- Identify the role of food products on human illness, risky behaviours and vulnerable populations;
- Contribute information for the comparison of interventions, direct actions and advance public health policy related to foodborne infections;
- Assess the effectiveness of food safety activities and public health interventions and measure performance.

In order to be captured by a surveillance system, a person must seek medical care, be requested to submit a clinical sample, must provide a clinical sample for testing, a test has to be successfully performed by private/hospital laboratories and a report submitted to the provincial/territorial/national levels. This complex chain of activities sets significant limitations on the ability of any surveillance system to identify case, which contributes to the under-reporting of foodborne illness. In addition, the collection of surveillance data can be performed through different mechanisms, depending on the resources available, ranging from no formal surveillance systems to a fully integrated food safety continuum surveillance system. In circumstances where no formal system is in place, the identification of cases may be based on absences from schools, illnesses after an event or within a closed environment (e.g., long term care centre), or other community measures. Although this might identify outbreaks or clusters of disease, it will not provide information on trends or illnesses occurring outside of these groups, nor will it identify the causative organism. Similarly, a syndromic surveillance system based on standard case definitions or pharmaceutical sales does not collect information on the organism that is causing disease or any demographic or exposure information related to the case but can provide limited trend information.

National monitoring of VTEC in Canada is conducted through different surveillance systems coordinated by the PHAC. It is recognized that most cases of acute diarrhoea are self-limiting, but there are certain circumstances that require the testing of samples, particularly for public health reporting purposes. In 2011, guidelines on when to test and treat cases of infectious diarrhoea were published in the Canadian Medical Association Journal (Hatchette and Farina, 2011), including guidelines for testing potential VTEC cases. The published guidelines recommend that patients presenting with non-bloody diarrhoea should



be tested to detect *E. coli* O157 only if the patient resides in a closed facility (e.g., long-term care facility), is a daycare worker, food handler or health care worker. In addition, the presence of dehydration, fever or underlying comorbidities among cases with non-bloody diarrhoea would also lead to testing for VTEC O157. All patients presenting with bloody diarrhoea are to be tested for *E. coli* O157.

Unfortunately, diagnosis and monitoring of non-O157 VTEC was hampered by the absence of standardized guidelines for when or how to test for these pathogens. However, in 2018, National guidelines to address this lack of standardization were published by the Canadian Public Health Laboratory Network (Chui et al., 2018). Recommendations include the use of either a chromogenic agar culture or a culture-independent diagnostic test (CIDT) for screening stool samples. If a CIDT method is positive for VTEC, laboratories should be able to culture and isolate VTEC in order to support surveillance and outbreak response. The overall objective of these recommendations is to improve detection of VTEC in patients presenting with diarrhoea, particularly when caused by non-O157 serotypes.

### **6.1 National Enteric Surveillance Program**

The National Enteric Surveillance Program (NESP) is a national surveillance system jointly administered by the PHAC's National Microbiology Laboratory (NML) and the Centre for Foodborne, Environmental and Zoonotic Infectious Diseases (CFEZID) since 1997 ([NESP](#)). It currently collects information on 14 different bacterial, viral and parasitic enteric pathogens. NESP's main objectives are to detect and respond to multi-jurisdictional outbreaks through weekly analysis and reporting of laboratory confirmed enteric disease cases in Canada; and to integrate with national and international efforts to monitor and limit the spread of enteric diseases. The collection of weekly enteric disease data allows for timely reporting and provides PHAC and provincial/territorial partners with the first signals that a significant disease trend is emerging. The collection of baseline data on enteric disease in Canada also facilitates understanding of trends in organism subtypes over time and supports research, inter-provincial and international comparisons, and planning and decision making for laboratorians, epidemiologists and other public health stakeholders.

NESP is a passive surveillance system relying on provincial public health laboratories to provide weekly reports for organisms isolated from Canadians that sought medical attention. All data is provided to NESP in an aggregate and anonymous manner and includes only the number of isolates from new cases identified at the provincial laboratory that week, or provides updates to previously reported numbers. Data analysis is conducted on the weekly data by PHAC to determine if case counts are significantly "higher than expected" based on a 5-year moving average. NESP weekly reports are produced and are only available to health professionals involved with monitoring enteric disease, working at the federal, provincial/territorial and regional/local levels with a public health mandate. NESP also produces Annual Summary reports, which are published on-line ([NESP Reports](#)).

The NESP determined rate of VTEC illness for Canada in 2016 was 1.7 per 100,000, of which 1.1 per 100,000 can be attributed to VTEC O157 and 0.6 per 100,000 to other VTEC serotypes (Government of Canada, 2018). From 1997 to 2007, the national rate of VTEC O157 illness in Canada was in the range of 3 to 5 per 100,000 (Figure 4). Following 2007, the rate of VTEC O157 has declined and remained stable around 1.2 per 100,000. In contrast the rate of non-O157 VTEC has steadily increased since 2011. There is also considerable regional variation in reported incident rates of VTEC, with Nunavut, Prince Edward Island and the Yukon reporting significantly greater incidence rates than the national rate in 2016.



Due to limitations in laboratory testing and reporting, there are inherent limitations to the data provided and presented by NESP. Therefore, the numbers reported in the provincial reports and the NESP data are an under-representation of the true incidence of disease in Canada. While VTEC O157 is routinely forwarded to provincial or central reference laboratories for further subtyping, non-O157 cases are poorly captured by NESP and are under-represented. With the introduction of whole genome sequencing and the development of new testing guidelines for the identification of non-O157 cases, it is expected that this limitation will be reduced and that cases reported to NESP will be closer to the true incidence of this disease in Canada.

## **6.2 PulseNet Canada**

Led by the PHAC's National Microbiology Laboratory (NML), PulseNet Canada is the national real-time molecular subtyping network for foodborne disease surveillance ([PulseNet](#)). The laboratory network has been in operation at PHAC since approximately 2000, with comprehensive real-time data being collected and analyzed on a daily and weekly basis since 2004.

The objectives of PulseNet Canada are to:

- Collect molecular and genomic subtyping data from cases of bacterial foodborne disease from all provincial public health laboratories, and from bacterial pathogens isolated by the CFIA, in real-time;
- Analyze these molecular data on a daily basis for the purpose of detecting potential outbreaks, particularly multijurisdictional outbreaks as early as possible; and to
- Provide the laboratory investigation during multijurisdictional outbreak response and support for single jurisdiction response to enable timely public health action to protect the health of Canadians.

PulseNet Canada has links to other PHAC surveillance activities, specifically, NESP and FoodNet Canada surveillance systems. Each of these surveillance systems collects data on foodborne pathogens, with PulseNet Canada focusing on the detection of potential outbreaks by identifying clusters of isolates with shared subtyping profiles. By capturing strain-level data, PulseNet compliments the species or serotype level data captured by NESP. PulseNet Canada data is analyzed jointly with NESP on a weekly basis. Currently, PulseNet Canada is carrying out whole genome sequencing for all VTEC isolates and performing cluster analyses on a weekly basis. If available, food, animal and environmental samples are included in these weekly analyses.

## **6.3 FoodNet Canada**

FoodNet Canada (FNC) is a national food safety "sentinel site" surveillance system facilitated by PHAC ([FoodNet Canada](#)). FNC activities are intended to integrate human, food, and environmental monitoring. The Region of Waterloo, Ontario was selected as FNC's first sentinel pilot site with surveillance activities beginning in 2005. In 2010, a second sentinel site was established within the Fraser Health Authority region, in British Columbia. In 2014, a third sentinel site was launched in the Calgary and Central zones of Alberta. Also in 2014, the Ontario site moved from Waterloo Region to the Middlesex-London Public Health unit area. Each sentinel site is a geographically defined area where samples from the local public health unit, retail food outlets, farms and local water sources are linked to generate information that is representative of the broader population.

FoodNet Canada's primary objectives are to:

- Determine what food and other sources are making Canadians ill;
- Determine significant risk factors for enteric illness;
- Accurately track disease rates and risks over time;

- Provide practical prevention information to prioritize risk mitigation/risk management efforts; compare interventions, direct actions and advance policy; and assess effectiveness of food safety activities and public health interventions and measure performance.

FoodNet Canada conducts a combination of active (farm, retail and water components) and passive (human) surveillance. Using a standardized questionnaire, epidemiological information is collected on every illness case and linked to laboratory results from work performed by provincial laboratories or NML. On a weekly basis, grocery stores are visited in the sentinel sites to purchase samples of raw meat and seafood available to the consumer. Samples are tested for the presence of several targeted enteric pathogens that are similar to those tested in food animals and water, including VTEC. Untreated surface water samples are collected bi-weekly and are tested for the presence of several targeted enteric pathogens and water quality indicators are recorded. The active surveillance of food animals is accomplished through the analysis of manure sampled on farms within each site, including fresh fecal matter, stocked manure or slurry. Sampling is conducted in four commodity groups with each farm visited once a year: swine, dairy, beef, and broiler chicken operations. Laboratory results are linked with data provided through a sample collection form.

All data is integrated to compare pathogens found in retail food, water and on farms with human infections to help identify what food and other sources are making Canadians ill. Data is further analysed to produce an annual report (FoodNet Canada Publications). Results from the food animal surveillance component are disseminated to each producer through an individualized report of their results on a yearly basis. Additional analyses are performed upon request by the sentinel sites, CFEZID-Outbreak Management Division, or federal partners. A summary of FoodNet VTEC test results for the period 2014 to 2017 is provided in Tables 6 and 11.

All human VTEC isolates recovered under FNC activities are characterised in the PulseNet Canada system, currently this includes WGS, which provides further subtyping information to differentiate among the non-O157 subtypes. VTEC isolates recovered from retail, farm or environmental samples are also sequenced and shared with PulseNet Canada. The inclusion of these non-human isolates allows for the detection of possible sources of infection, transmission routes of the pathogen along the food chain, and informs the generation of hypothesis for outbreak investigations.

#### **6.4. Summary**

National monitoring of VTEC illness in Canada is conducted through three programs, the National Enteric Surveillance Program, PulseNet Canada and FoodNet Canada. The primary features of these programs are:

- Information on the rate of VTEC illness in Canada is captured by the NESP, based on data provided by provincial public health laboratories. This data allows reporting of incident rates and identification on changes in incident trends.
- Due to limitations in laboratory testing and case reporting, NESP incident rates of VTEC are understood to be an underestimate.
- The incident rate of VTEC illness for Canada in 2016 was 1.7 per 100,000. Incident rates have been relatively stable since 2008, declining from 3 to 5 per 100,000 in the previous decade.

- Incident rates of VTEC vary regionally. For example, Nunavut, Prince Edward Island and Yukon reported significantly greater incidence rates than the national rate in 2016.
- PulseNet Canada conducts whole genome sequencing for all VTEC isolates available, including isolates from clinical, food, animal and environmental samples. To support the identification of potential outbreaks and exposure sources cluster analyses is conducted to identify related isolates.
- FoodNet Canada conducts active surveillance at three sentinel sites: the Fraser Health Authority region, in British Columbia, the Calgary and Central zones of Alberta, and the Middlesex-London Public Health unit area in Ontario. To identify causes and sources of foodborne illness FoodNet collects and tests samples from the local public health unit, retail food outlets, farms and local water sources for enteric pathogens, including VTEC.

## **7. Monitoring VTEC in Food**

In addition to the surveillance systems designed to monitor human health, Canada has a national food safety system that is designed to protect the health of consumers and ensure fair practices in the food industry according to the principles recommended by the Codex Alimentarius (CAC, 2013). Within this system, food monitoring programs are used to (i) provide information on specific hazards in foods, (ii) contribute to the development of risk management options, and (iii) support system-wide review activities.

Food monitoring programs contribute to hazard identification and provide data to inform quantification of exposure. Monitoring may be conducted to support compliance verification, standard development and to demonstrate country equivalency as well as to assess the effectiveness of implemented risk management measures during monitoring and system review. A limitation of microbial food surveillance data is that the foodborne pathogens may be present at very low frequency in foods. Thus, unless very large numbers of samples from a population are tested, it may be difficult to assess the prevalence of these pathogens with a high degree of certainty.

### **7.1 Federal VTEC Monitoring/Testing Programs**

#### **National Microbiological Monitoring Program and Food Safety Oversight Program**

The CFIA conducts two monitoring programs which include testing foods for VTEC, these are the National Microbiological Monitoring Program (NMMP) and the Food Safety Oversight (FSO) Program. The NMMP is primarily focused on federally registered production facilities and is dependent upon inspector sampling at registered establishments. In the FSO Program, which was recently introduced to complement the NMMP, samples are collected by both CFIA inspectors and third-party samplers at registered establishments and at retail stores. Results from both programs are published annually on the CFIA website (CFIA [Testing](#)).

#### **Targeted Surveys Program**

The CFIA also performs Targeted Surveys to examine hazards and/or foods that are not routinely included in other CFIA monitoring programs. Unlike NMMP and FSO, which monitor established food hazard combinations over time, the foods included in Targeted Surveys may vary from year to year. From April 1, 2013 to March 31, 2018, six types of foods were sampled and tested for VTEC (Table 7): fresh and fresh-cut RTE fruit and vegetables, raw milk cheeses, raw ground pork, beef and veal, nuts and nut butters, dried sprouted seeds and cold-pressed/ unpasteurized juices and ciders (Table 7).

## **FoodNet**

As discussed in section 6.3 FoodNet Canada provides integrated human, food, and environmental monitoring within designated “sentinel sites”. Sampling of ground beef at retail is a core activity under FoodNet Canada and is included every year. Depending on public health concerns identified by partners, additional sampling of other targeted products is conducted. In recent years, targeted sampling has included ground pork, veal or pork sausage. FoodNet Canada presents their surveillance data through their annual reports ([NESP Reports](#)).

## **7.2 Results of Federal VTEC Monitoring/Testing Programs in Food**

### **7.2.1 Raw Ground Beef, Pork and Veal and Precursor Materials**

Under the NMMP, finished raw ground beef or veal products (FRGBP) and precursor materials are tested for *E. coli* O157:H7/NM. Sampling of FRGBP is performed at the first grinding phase (coarse grind) of trims and other precursor materials of selected production lots of a minimum 900 kg. Precursor materials intended for use in FRGBP included trims, boneless beef, coarse ground beef, hearts, head meat, cheek meat, tongue roots, weasand meat, and chucks. The frequency of sampling at domestic establishments is based on the size of the establishment and its compliance history. Sampling frequency is higher in the summer. From April 1, 2013 to March 31, 2018, *E. coli* O157:H7/NM was isolated from 0.09% (n = 3,273) of domestic FRGBP samples and in 0.13% (n = 3,885) domestic samples of FRGBP precursor (Table 7).

FoodNet testing of retail ground beef over 4 years (2014 to 2017) had a VTEC isolation rate of 2.0% (n = 1458). The frequency of VTEC in retail ground pork was 6.1% (n = 98) in 2014 and 2015. A similar frequency of VTEC 6.3% (n = 334) was reported for retail veal in 2017 (Table 6).

### **7.2.2 Ready-to-Eat Meats**

Under the NMMP, ready-to-eat (RTE) meats, that have not been fully cooked, such as some dry, semi-dry or fermented products, are tested for *E. coli* O157:H7/NM. No VTEC were isolated from 22 domestic RTE meat samples and 15 imported RTE meat samples tested under the NMMP from April 1, 2013 to March 31, 2018 (Table 7).

### **7.2.3 Raw Milk Cheeses**

Under the NMMP, raw milk cheeses, including cow, goat and sheep’s milk cheeses, were tested for *E. coli* O157:H7/NM. No VTEC were isolated from 247 domestic raw milk cheese samples and 550 imported raw milk cheese samples tested under the NMMP from April 1, 2013 to March 31, 2018 (Table 7).

### **7.2.4 Fresh Produce**

Under the NMMP and FSO, fresh and fresh-cut RTE fruits and vegetables are tested for VTEC. Fresh fruits and vegetables include imported whole, fresh leafy vegetables, tomatoes, fresh herbs, green onions, peppers, sprouted seeds and beans, cantaloupes, papayas, mangoes and berries. Fresh-cut RTE fruits and vegetables are defined as fresh fruits or vegetables that were washed and further minimally processed (e.g., peeled, cored and sliced, chopped and/or shredded) prior to being packaged. Also included are pre-packaged salads. Testing is currently for *E. coli* O157:H7/NM, although some fresh produce was tested for non-O157 VTEC in the past. No *E. coli* O157:H7/NM were isolated from 2617 domestic fresh and fresh-cut RTE fruits and vegetable samples and 4882 imported fresh and fresh-cut RTE fruits and vegetable samples tested under the NMMP and FSO from April 1, 2013 to March 31, 2018 (Table 7). No *E. coli* O157:H7/NM or non-O157 VTEC were isolated from 66 domestic fresh and fresh-cut

RTE fruits and vegetable samples and 187 imported fresh and fresh-cut RTE fruits and vegetable samples tested under the NMMP from April 1, 2013 to March 31, 2018 (Table 7).

Under Targeted Surveys, fresh and fresh-cut RTE fruit and vegetable products tested included fresh herbs, leafy vegetables, green onions, cucumbers, sprouted seeds and beans, mangos, papayas, and stone fruits (peach, plum, nectarine, apricot, etc.). Fresh-cut RTE fruits and vegetables included pre-packaged RTE vegetables and fruits (e.g., berries, mangos, pineapple), including melons (e.g., watermelon, honeydew melon, and cantaloupe). No *E. coli* O157:H7/NM were isolated from 28,715 domestic and imported fresh and fresh-cut RTE fruits and vegetable samples tested for *E. coli* O157:H7/NM under the Targeted Surveys from April 1, 2013 to March 31, 2018 (Table 7). Six non-O157 VTEC were isolated from 1251 domestic and imported fresh and fresh-cut RTE fruits and vegetable samples tested for *E. coli* O157:H7/NM and non-O157 VTEC under the Targeted Surveys during the same time period (Table 7).

### **7.2.5 Other Plant-Based Foods**

In addition to the foods listed above, Targeted Surveys were performed on nuts and nut butters, dried sprouted seeds and cold-pressed/ unpasteurized juice and cider samples between April 1, 2013 and March 31, 2018 (Table 7). No VTEC were isolated from 3,972 domestic and imported nut and nut butter samples tested for *E. coli* O157:H7/NM. No VTEC were isolated from 322 domestic and imported dried sprouted seeds tested for *E. coli* O157:H7/NM but 0.4% (n = 1,028) domestic and imported dried sprouted seeds tested for *E. coli* O157:H7/NM and non-O157 VTEC were found to contain non-O157 *E. coli*. Lastly, no VTEC were isolated from 1,133 domestic and imported cold-pressed/ unpasteurized juice and cider samples tested for *E. coli* O157:H7/NM.

### **7.3 Industry VTEC Monitoring/Testing**

Abattoirs producing raw beef trimmings for export to the USA participate in a CFIA-designed verification sampling program. Participation in this program is mandatory for abattoirs to maintain their eligibility to export to the USA. Samples are collected by the operators under the supervision of the CFIA veterinarian in charge and are submitted to private laboratories for analysis for the presence of LEE-positive VTEC belonging to the serogroups O26, O45, O103, O111, O121, and O145.

The private laboratories are required to use only test methods that have been granted a No Objection Letter (NOL) from the United States Department of Agriculture (USDA) Food Safety Inspection Services (FSIS) for the screening for the presence of the targeted VTEC. All results obtained under this program, i.e., negative, presumptive positive and culturally confirmed positives, are reported by the operator's contracted laboratory to the CFIA.

This program complements *E. coli* O157:H7/NM testing that is performed by the CFIA under its NMMP, as sampling frequencies for both industry and CFIA testing are the same and the same robust N60 sampling approach is used for both.

### **7.4 Summary**

Surveillance data for VTEC in foods is collected through the CFIA National Microbiological Monitoring Program and Food Safety Oversight Program, which are supplemented by Targeted Surveys of selected products. Data on VTEC contamination of retail ground beef, pork and veal is collected through FoodNet Canada. The primary findings of these surveys are as follows:

- Foods for which surveillance data is available include; raw ground beef, pork, and veal, ground meat precursor materials, ready-to-eat meats, raw milk cheeses, fresh produce, nuts and nut butter, dried sprouts, cold-pressed/unpasteurised apple juice. Both domestic and imported products have been surveyed. VTEC were isolated from raw ground meats, fresh produce and dried sprouts, but not other foods tested.
- Domestic raw ground beef and veal was positive for VTEC O157 at a prevalence of 0.09% (n = 3,273) for 325 g samples.
- Domestic raw ground beef precursor was positive for VTEC O157 at a prevalence of 0.13% (n = 3,885) for 325 g samples.
- VTEC isolation rate from retail ground beef 2.0% (n = 1458), retail ground pork 6.1% (n = 98) and 6.3% (n = 334) for 25 g samples.
- A total of 36,214 samples of fresh and fresh cut ready-to eat vegetables (25 g or 125 g) of domestic or imported origin was tested for VTEC O157 and was found negative. Non-O157 VTEC was isolated at a rate of 0.40% (1,504).
- The prevalence of non-O157 VTEC in dried sprouts was 0.39% (n = 1,028) for 25 g samples.

## **8. Analytical Methodology for VTEC**

### **8.1. Food Testing Methods**

Due to the high infectivity of VTEC, there is no established safe exposure to VTEC; thus, methods for the analysis of foods for the presence of VTEC are typically qualitative and composed of the following stages: Enrichment, Detection, Isolation and Confirmation. Enrichment involves the incubation of the sample in broth media to amplify the target pathogen to levels high enough for reliable Detection and Isolation. This stage is necessary, as, due to the high infectivity of some VTEC strains, a limit of detection approaching 1 cell per analytical unit (10 g to 375 g) must be achievable. Detection is an optional stage in which the presence or absence of biomarkers specific for the pathogen in the enrichment broth is determined. Detection analysis typically involves PCR or serological tests. If pathogen-specific biomarkers are not detected, the sample is considered negative and analysis can be terminated; if pathogen biomarkers are detected, the sample is presumptive positive for the pathogen. Isolation involves the recovery of presumptive pathogen cultures from the enrichment media and purification of that pathogen from other organisms present. This step is typically performed by plating on to agar media and provides pure cultures for Confirmation testing. The presence of the pathogen in the sample is confirmed by determination that one or more isolates recovered from the sample possesses the specific characteristics of the pathogen. For VTEC, this is the isolation of *Escherichia coli* with the potential to produce verotoxin, which can be determined either by production of the toxin or the presence of its genes. The isolates identified are then available for further characterisation, including subtyping.

Due to the mobility of the phage encoded verotoxin genes, VTEC do not constitute a discrete phylogenetic lineage among *E. coli*, and there are no phenotypic characteristics unique to VTEC, such as antimicrobial resistance or metabolism, which can form the basis for differential/selective media. However, features specific to certain VTEC subpopulations have been identified and form the basis of

analytical methods targeting those subgroups. Methods for VTEC O157:H7/NM can take advantage of relatively high resistance to certain antimicrobials (novobiocin, tellurite, and cefiximine) to increase media selectivity and differentiation based upon the absence of sorbitol fermentation and  $\beta$ -D-glucuronidase activity and the presence of haemolysis on washed sheep's blood agar (Beutin et al., 1989; March and Ratnam, 1986; Doyle and Schoeni, 1984). Similarly, the absence of rhamnose fermentation can be used as a differential feature for some O26 strains (Murinda et al., 2004). It should be noted that variability in colony morphology and other phenotypic traits can be observed between strains of the same serogroups, including *E. coli* O157 (Werber et al., 2011; Gill et al., 2014).

In some jurisdictions, methods of analysis have been adopted which target a subgroup of priority VTEC defined by serogroup and the presence of intimin (*eae*), for example, the USDA FSIS method MLG 5B (USDA-FSIS, 2018) and the European Union (EU) (ISO, 2012). The serogroups designated a priority in the US are O157, O26, O45, O103, O111, O121, and O145, and the current ISO method targets O26, O103, O111 and O121. MLG 5B utilises immunomagnetic separation (IMS), coupled with acid shock and elevated incubation temperatures to improve selectivity. However, this approach excludes VTEC belonging to other serogroups and LEE negative strains even though such strains have been responsible for foodborne outbreaks and serious illness. Additionally, low recovery rates have been observed with IMS for some targeted VTEC serogroups (Kraft et al., 2017; Hallewell et al., 2017) and the higher enrichment temperatures such as 42 °C may not be suitable for all strains, particularly cells that have been physiologically stressed.

Methods for the analysis of foods for VTEC validated for regulatory testing in Canada are published in *The Compendium of Methods of Analytical Methods* (Health Canada, 2018). These include commercial methods, the standard method of analysis for VTEC O157 (MFHPB-10) and the standard for all VTEC (MFLP-52) (Blais et al., 2014). The development of MFLP-52 was informed by the causal and correlative factors previously established through subject matter expert consensus (VTEC Workshop Report, 2010), and which may need to be updated in light of current public health trends. To ensure the inclusivity of the method the enrichment and isolation conditions represent a compromise between permitting the recovery of diverse VTEC strains, while imposing some selectivity against other microbiota (Gill et al., 2012; Gill et al., 2014). The MFLP-52 method for VTEC begins with broth enrichment, followed by PCR screening for *stx1* and *stx2*, if the enrichment is positive for one or more verotoxin genes the sample is presumptive positive. Presumptive positive enrichments are then plated onto agar media and following incubation up to 60 individual colonies are screened by PCR for *stx1* and *stx2* to identify VTEC (Blais et al., 2014). Confirmation involves a Cloth-based Hybridization Array System (CHAS) for detecting PCR amplicons for the virulence genes *stx1* and *stx2*, *eae*, *hlyA* and serogroup markers for O26, O103, O111, O145, and O157. This approach is intended to be inclusive while supporting rapid identification of markers associated with VTEC causing BD and HUS.

The greatest potential for the application of next generation sequencing (NGS) to the analysis of food for VTEC is the genomic characterisation of isolates. As the cost and speed of NGS has fallen the technology has become economically competitive with other methods of isolate characterisation particularly as once a genome has been sequenced the same data can be interrogated for the presence of multiple elements or subjected to multiple forms of subtyping analysis. An additional advantage is a greater flexibility in the analysis of isolates: rather than relying solely on rigidly validated “wet lab” methods, NGS analysis can be adapted *ad hoc* for the determination of gene markers that may be relevant to a particular food safety event, or in response to criteria indicated by changing public health trends. Regulatory agencies in the international community have been actively developing strategies for the implementation of WGS technology in support of regulatory food inspection objectives through the

detection, identification and characterization of priority bacterial pathogens such as VTEC (Tong et al., 2015; Lambert et al., 2015; Lambert et al., 2017, Carrillo et al., 2019).

CFIA laboratories have developed a practical process in which genomic DNA isolated from single colonies is sequenced using the Illumina MiSeq platform, followed by analysis of the sequence data in a fully integrated process for the determination of key genomic markers (Lambert et al., 2015; Blais, 2017). A bioinformatics pipeline named GeneSeekR has been designed to determine salient features such as identity (e.g., species, serotype), risk characterization attributes (e.g., virulence, verotoxin subtypes), molecular type (single nucleotide polymorphism and multi-locus sequence typing analyses) and “value-added” markers (e.g., antibiotic resistance profile for surveillance purposes). Quality assurance metrics are included to indicate the reliability of each analysis, including indicators for sequence data quality and bioinformatics performance. The recently developed ConFinder tool (Low et al., 2019) is designed to identify the presence of contaminating DNA from multiple strains of the same bacterial species. A key output is the Record of Genomic Analysis (ROGA) featuring a standardized reporting format intended to meet the needs of the end-user community (i.e., Food Safety Science Directorate, risk assessors and recall specialists). This technology is currently being used by CFIA to generate WGS data for food isolates in real time. The implementation of this new program provides an unprecedented degree of resolution in the analysis of foodborne bacterial isolates, enabling their timely identification and risk profiling at a cost similar to traditional methods, and has the potential to replace lengthy biochemical characterization and typing procedures used in contemporary food-testing laboratories.

A key capacity afforded by the WGS approach is the ability to rapidly identify important attributes associated with risk, such as the serotype and verotoxin subtypes of VTEC isolates, without the need to forward isolates to specialized reference centres for lengthy analytical procedures. Serotype can be readily determined using the SeroTypeFinder tool (Joensen et al., 2015) hosted by the Centre for Genomic Epidemiology ([www.genomicepidemiology.org](http://www.genomicepidemiology.org)). Currently, the most widely used standard method for verotoxin subtyping is a PCR technique developed by the Statens Serum Institute (SSI), which differentiates subtypes using subtype-specific primer pairs (Scheutz et al., 2012). More recently, a validated *in silico* technique based on WGS analysis using an algorithm simulating the SSI PCR process, termed V-Typer, has been incorporated in a bioinformatics pipeline routinely used at CFIA for the characterization of VTEC isolates (Carrillo et al., 2016).

An emerging application of WGS technology is the use of genomic information to inform tailoring of methods of analysis to improve the probability of recovery of specific pathogen strains. For example, it has been demonstrated that investigation of foodborne VTEC outbreaks can be supported by the prediction of antimicrobial resistance of the pathogen strain from genomic data to indicate antimicrobial agents which can be used to customise the selectivity of culture media (Knowles et al., 2016; Blais et al., 2019). AMR analyses can be conducted with the use of publicly available tools such as ResFinder (<https://cge.cbs.dtu.dk/services/ResFinder/>). Such an approach has been demonstrated to be effective in the recovery of different VTEC strains from ground beef samples containing high levels of background bacteria (Blais et al., 2019). When applied in outbreak investigations for the detection and isolation of specific VTEC strains, this approach can potentially overcome the limited selectivity of the current enrichment conditions.

## **8.2. Clinical Methods**

While testing of VTEC O157 is a routine practice for local, private and hospital laboratories, there are no standardized guidelines for when to test for other VTEC serotypes. In late 2016 and early 2017, Canada experienced an outbreak of VTEC O121 due to contaminated flour (Morton et al., 2017). Due to the lack



of standardized guidance on when to test for non-O157 VTEC, concerns regarding missing cases that were part of this investigation were raised by provincial public health laboratories and epidemiologists. As such, interim guidelines were provided to public health laboratories on when to test for non-O157 VTEC to ensure that cases associated with this outbreak were identified. National guidelines to address this lack of standardization have been published by the Canadian Public Health Laboratory Network (Chui et al., 2018).

A challenge to monitoring VTEC in Canadians is the introduction of the use of culture-independent diagnostic tests (CIDT) to diagnose foodborne infections. While traditional methods require the isolation of a foodborne organism from a specimen sample submitted by a patient, these rapid tests identify the causative organism but do not result in an isolate for further subtyping (e.g., identification of serotype) or testing (e.g., antimicrobial susceptibility). As the uptake of CIDT increases, surveillance systems in Canada and internationally will experience erosion of the capacity to conduct surveillance of foodborne pathogens. Particularly vulnerable is the granularity required to distinguish between sporadic and outbreak associated cases. A proposed solution to the increased use of CIDT is conducting reflexive culture – using a CIDT for a panel of enteric pathogens and then culturing positive samples, in order to obtain isolates. These isolates would then be available for further testing and to inform surveillance systems. However, in countries where the use of CIDT has become prevalent and reflex culture has been introduced, several roadblocks have been identified in making this approach a success. The primary issue identified is the cost of the additional laboratory work required for culture and the lack of clarity on who is responsible for covering this cost. If physicians are not requesting cultures to be performed, the costs associated with adding this test are not reimbursed to the local laboratories. If local laboratories do not culture the samples, then these will be required to be sent to public health laboratories, adding a level of complexity due to shipping timelines and costs.

### **8.3. Summary**

- Methods of analysis for VTEC in food typically have four parts: Enrichment in broth media; Detection of biomarkers for VTEC; Isolation of VTEC from the enrichment; and Characterisation of the VTEC isolates.
- Due to the high infectivity of VTEC and the potentially low levels in contaminated foods, methods of analysis for VTEC in foods must have a limit of detection approaching 1 cell per analytical unit, which may be as large as 375 g. To achieve this sensitivity amplification of VTEC by enrichment is a necessity.
- Isolation of VTEC is necessary to confirm that the virulence markers detected in enrichment represent a viable cell and provide isolates for characterisation.
- Enrichment and Isolation remain challenging as there is currently no selective or differential media available which is specific for VTEC. VTEC are not members of a discrete phylogenetic lineage and so there are no phenotypic characteristics unique to VTEC which can form the basis for differential/selective media.
- There are phylogenetic groups within the VTEC which can be selected/differentiated on the basis of antimicrobial resistance or substrate utilisation.

- Methods for the analysis of foods for VTEC validated for regulatory testing in Canada are published in The Compendium of Methods of Analytical Methods. The standard method of analysis for VTEC O157 is MFHPB-10 and the standard for all VTEC is MFLP-52.
- Next generation sequencing offers numerous advantages and cost savings in the characterisation and subtyping of VTEC isolates.
- In Canada, clinical testing for VTEC O157 is a routine practice. Clinical testing for non-O157 VTEC is more challenging and there has been a lack of established standards. Recently national guidelines to address this lack of standardization have been published by the Canadian Public Health Laboratory Network.
- The increasing use of culture-independent diagnostic tests (CIDT) to diagnose clinical cases of infection by VTEC and other foodborne pathogens may erode public health surveillance. As CIDT does not provide either isolates or data from which subtyping can be conducted to identify clusters or conduct source tracking.

## **9. Risk Mitigation**

Regardless of the food commodity, intervention-based Hazard Analysis and Critical Control Points (HACCP), HACCP-like or Good Agricultural Practices/Good Manufacturing Practices (GAP/GMP) are recommended and frequently in place at various points in the food production and processing environment continuum to control microbiological hazards that may be present; this includes VTEC. Historically, in Canada, the major hazard considered in this category has been VTEC O157:H7/NM. Regardless, there is no evidence that non-O157 VTEC and VTEC O157 are significantly different in their resistance to environmental stresses and decontamination processes currently in place in food processing (Kundu et al., 2014; Liu et al., 2015; Gill et al., 2019b). Thus, it can be expected that those systems which effectively control VTEC O157:H7/NM will also control other VTEC.

Healthy cattle and other domestic ruminants are known to be carriers of VTEC, which can be transferred to meat and milk during harvest. A wide range of pre-harvest interventions to minimize the presence of VTEC O157:H7 in cattle have been investigated including: 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. While there is no intervention that has been demonstrated to prevent VTEC carriage in cattle or other ruminants or to reliably reduce VTEC shedding in feces, adherence to industry best practices with regards to cattle management and hygiene of food, water and transportation can help minimize VTEC along the food chain (Swaggerty et al., 2018).

### **9.1 Meat**

In Canada, the meat production sector is regulated and receives oversight from federal, provincial and municipal authorities. National baseline studies on the prevalence on VTEC O157:H7/NM have been conducted over the years and have helped in establishing inspection activities and domestic monitoring processing programs by regulatory authorities. No national level data (baseline study) is available at this time on the presence of non-O157 VTEC in domestic beef cattle. Food safety efforts, up to the present, have been largely focused on mitigating the risk from VTEC O157:H7/NM.

Beef processing is normally done in several stages, and various treatments (physical or chemical interventions) can be used along the processing chain to remove or inactivate pathogens if present. These interventions (e.g., chemical solutions, hot water treatment, etc.) can be applied at post-stunning, pre-evisceration and/or post-evisceration stages. The adoption of processing interventions at the major Canadian beef slaughter plants is correlated with a decline in the frequency of VTEC O157 contamination of beef and cases of human illness in Canada (Pollari et al., 2019).

To reinforce and enhance the application of preventative programs as well as help mitigate risk from VTEC O157:H7 a number of guidance documents have been issued at the federal level (Table 12.). Products which are the subject of guidance include: raw ground beef and precursor material; ready-to-eat fermented sausages, mechanically tenderized beef, and donair type meats.

### **9.2 Milk and Dairy Products**

Milk and dairy products producers and/or processors in Canada may receive federal or provincial oversight. Dairy farmers through the Dairy Farmers of Canada have adopted and implemented on-farm food safety program (the Canadian Quality Milk Program) that uses a HACCP based approach to help mitigate bacterial hazards, amongst others, in their products. Producers monitor critical areas relevant to food safety specifically: effective cooling and storage of milk, sanitation of equipment, and cleanliness of water.

Liquid milk must be pasteurized prior to sale in Canada (as per *Food and Drug Regulations*- FDR- (C.R.C., c. 870), B.08.002.2 (1)). It is expected that this treatment will control all strains of VTEC that might be present in raw milk. However, cheese made from unpasteurized milk is allowed for sale (B.08.002.2 (2a)).

### **9.3 Fresh Produce**

Fresh fruits and vegetables are frequently consumed in their raw state and thus represent a concern for public health because outbreaks involving these commodities often affect a large number of individuals.

In Canada, there are a number of programs aimed at proactively managing potential sources of contamination of fresh fruits and vegetables. Programs, such as the industry developed [CanadaGAP](#), are based on GAP as described by the Codex Alimentarius, with the aim of preventing microbiological contamination, minimizing public health impacts when contamination occurs and improving communication. The on-farm Food Safety Enhancement Program is a similar program, based on HACCP principles that complies with Federal, provincial/territorial requirements for food safety management (CFIA, 2018).

Specific guidance has been issued to help minimize the public health impact of contamination by bacterial pathogens of unpasteurized fruit juice/cider and sprouts from seeds and beans (Table 12).

## **10. Knowledge Gaps and Research Agenda**

In view of the foregoing considerations a number of important questions remain which might be answered through targeted research aiming to optimize regulatory responses to the occurrence of VTEC in the food supply:

### **VTEC Virulence Markers**

Verotoxin (*stx*, *vt*), intimin (*eae*), and aggregative adhesion (*aggR*, *aaiC*) have established roles in VTEC disease processes. What are the roles of other genomic markers in VTEC disease processes?

How should additional genomic markers be identified and interpreted?

What is the biological significance of *vt* and *eae* subtypes?

### **Subtyping of VTEC Isolates**

How should subtyping markers (serotype, wgMLST, etc.) be interpreted in evaluating VTEC isolates?

What are the objectives of routine subtyping and how is novel methodology determined to be “fit for purpose”?

Should historical data/isolates be sequenced and analyzed? How could this data be used for risk assessment and risk mitigation?

### **VTEC Exposure**

What is the significance of VTEC loads in various food commodities?

Considering the high infectivity of some VTEC, do low loads contribute to the sporadic distribution of cases?

### **Monitoring**

What is the true burden of human illness related to non-O157 VTEC infections in Canada?

Do current monitoring programs for VTEC in food provide the information required in a cost effective manner? Are there significant gaps in the products or sectors currently being monitored? Can information sharing be improved? What is the best approach for data and information sharing among multi-jurisdictional regulatory partners (e.g., leveraging established network initiatives such as the Canadian Food Safety Information Network, or creating new networks).

### **Methodology**

What methodological improvements are required? What is the best approach for the identification of risk markers, such as verotoxin subtypes or variants, using WGS techniques (e.g., assembly-dependent/-independent approaches, translation of sequence data and identification of definitive amino acids)?

What are the limitations of determining gene markers on the basis of sequence data given the occurrence of complex nucleotide polymorphism patterns and the discovery of possible novel variants?

What is the potential of predictive genomics in determining biological functionality and informing techniques for the recovery of VTEC? How should culture-independent testing be applied and the results assessed in the context of food safety investigations?

### **Standards and Guidance**

Is there a need to develop new or update standards and guidance related to VTEC in foods?

Should such standards include serotype? What virulence markers should be used?

## **11. Conclusions**

While many questions remain to be answered regarding the factors defining VTEC of public health concern, the best information available at this time indicates a possible way forward in assessing food-borne VTEC isolates for food safety purposes.

- The current best definition of VTEC of public health concern at this time includes, but is not limited to, the presence of key virulence markers including all VT1 and VT2 variants, key adhesion genes such as *eae* and/or *aggR* (and/or possibly others, which will need to be clearly defined).
- Food testing methods should be able to rapidly discriminate O157:H7 VTEC from non-O157 VTEC as the former is immediately actionable on primary identification, while the latter may be comprehensively characterized by high-capacity techniques including (but not limited to) WGS analyses to determine the serotype, VT subtypes, sequence type, and other salient features which need to be considered in conjunction with other relevant information linked to the risk assessment process. Notwithstanding, it is important to understand and consider the limitations of NGS technology in interpreting results.
- VTEC are a complex family of pathogens created through the transfer of mobile genetic elements. There is significant variability in the presence of specific virulence factors in individual strains of VTEC, so each isolate needs to be assessed on a “case-by-case” basis to determine its food safety significance. Such assessments may include consideration of all the pertinent factors described in this document.
- The scope of analysis and decision-making should not be limited to specific serogroups, since Canadian public health data show that infections with diverse serotypes may occur, often with strains falling outside the currently defined “big seven” group of “priority” VTEC identified on the basis of US epidemiological data.
- More information is needed to clarify the role of different virulence genes and genetic variations reported in the literature, and there is a need to continue documenting WGS data for food and clinical VTEC isolates for information gathering to guide future refinements in the identification of VTEC of health concern and the attendant test approaches.
- Next generation sequencing genomics technologies are well suited for the analysis of food and clinical isolates in determining their salient characteristics for risk management purposes.

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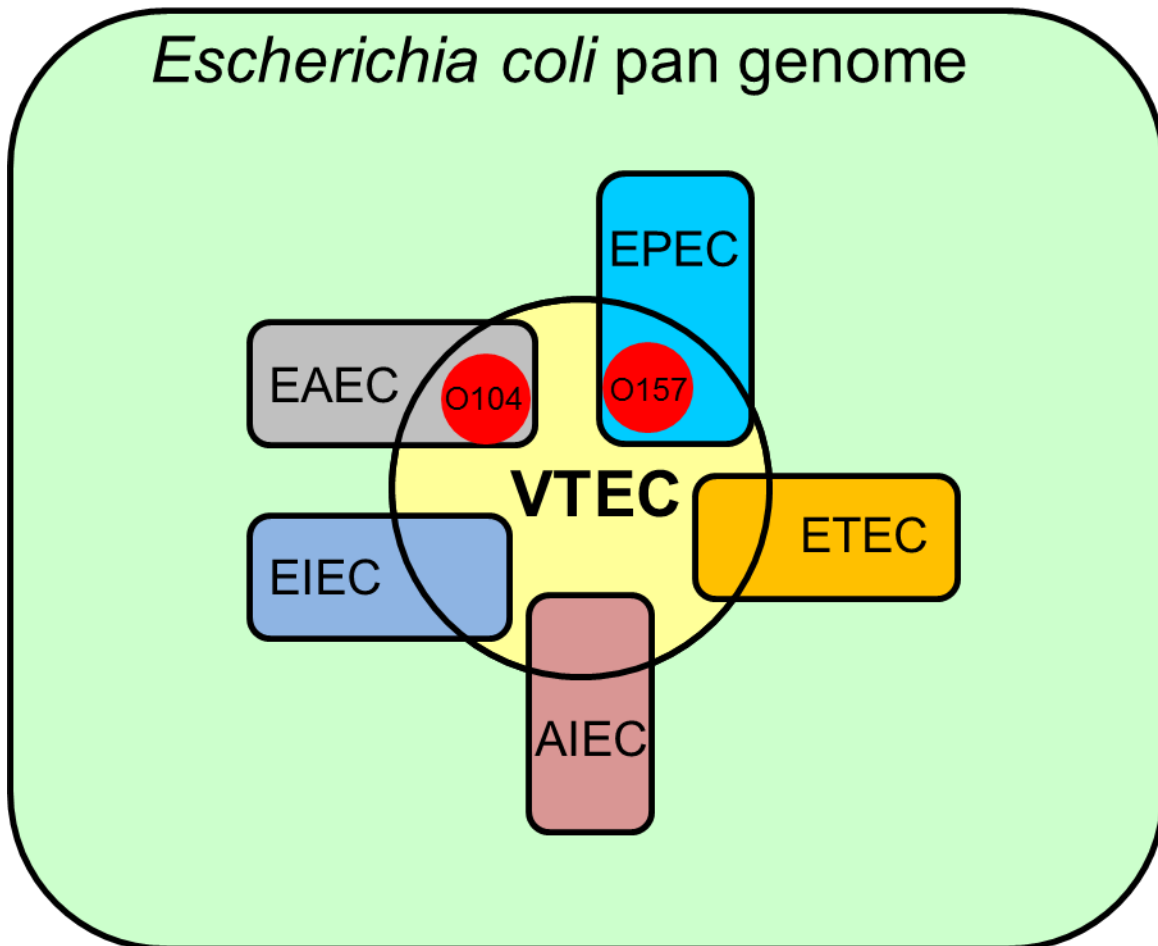
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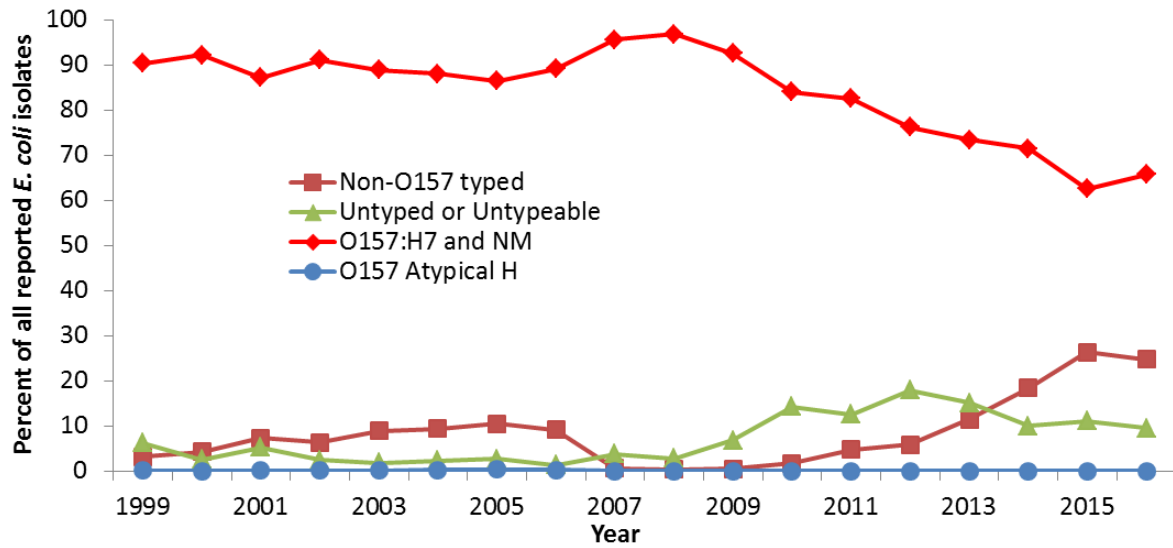


**Figure 1.** Relationship between the genomic content of verotoxigenic *Escherichia coli* (VTEC) and other *Escherichia coli* pathotypes. AIEC, adherent-invasive. EAEC, enteroaggregative. EIEC, enteroinvasive. EPEC, enteropathogenic. ETEC, enterotoxigenic. (Figure 1 is related to the **Hybrid Pathotypes** section)

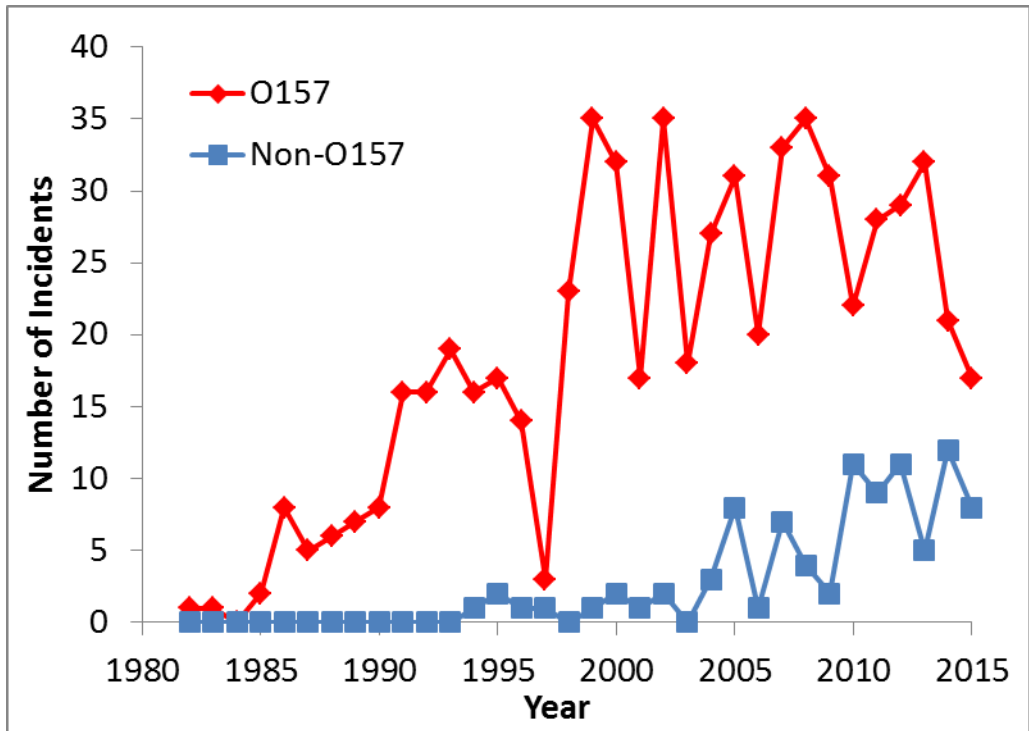




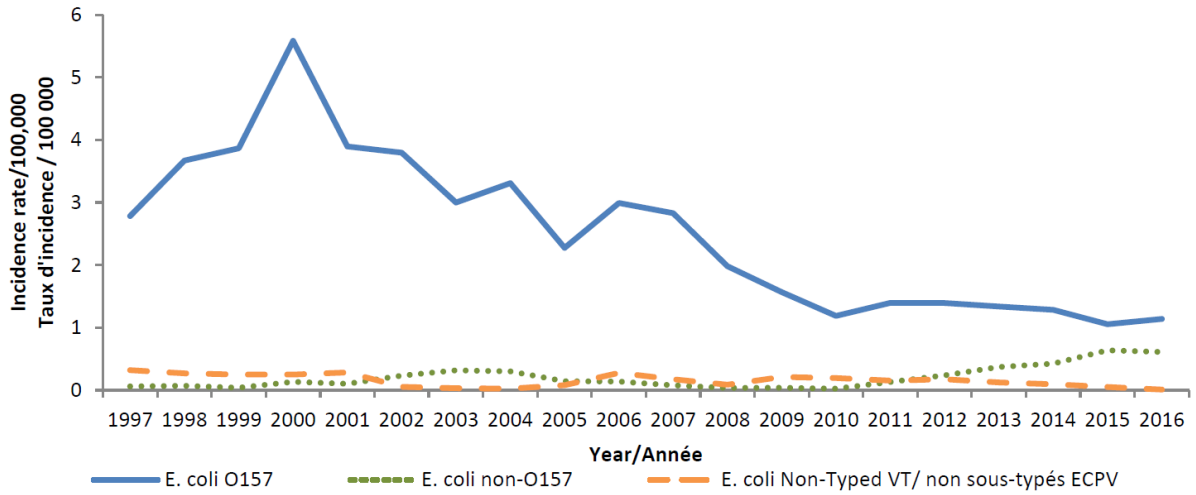
**Figure 2.** Percentage of all *Escherichia coli* isolates reported to PHAC NESP categorised by serotype, 1999 to 2016. NM, Nonmotile. (Figure 2 is related to the **Serotyping** section)



**Figure 3.** International and Canadian reports of foodborne Verotoxigenic *Escherichia coli* incidents with an identified food vehicle, 1982 to 2016. Data and citations in Supplement 2. (Figure 3 is related to the **Foods Associated with VTEC illness** section)



**Figure 4.** Canadian national incidence rate of verotoxigenic *Escherichia coli* (VTEC) O157, non-O157 VTEC and Non-Typed VTEC reported to NESP, 1997-2016. (Figure 4 is related to the **National Enteric Surveillance Program** section)



**Table 1.** Incidence of Patient Outcomes from confirmed verotoxigenic *Escherichia coli* infections US, 1996 to 2017. <https://www.cdc.gov/foodnetfast/>. (Table 1 is related to the **Features of Vulnerable Populations** section)

Age Group	Incidence per 100,000		
	Infection	Hospitalization	Death
<5	8.08	1.79	0.04
5-9	3.95	1.20	0.00
10-19	2.87	0.80	0.02
20-29	1.82	0.42	0.02
30-39	0.94	0.25	0.02
40-49	0.80	0.26	0.00
50-59	1.04	0.40	0.02
60-69	1.29	0.61	0.02
70+	1.48	0.87	0.06
Male	1.90	0.57	0.01
Female	2.19	0.66	0.01

**Table 2.** Virulence markers and putative virulence markers of verotoxigenic *Escherichia coli*. (Table 2 is related to the **Verotoxin** and **Other Virulence Factors** sections)

**The Content Of This Table Is Provisional.**

Target	Confirmed Virulence Factor	Genetic Support	Encoded protein or family effector	GenBank Accession Numbers
<i>stx1a</i>	Yes	Chromosomal -phage	Verotoxin 1a	M19473
<i>stx1c</i>	Yes	Chromosomal -phage	Verotoxin 1c	Z36901
<i>stx1d</i>	Yes	Chromosomal -phage	Verotoxin 1d	AY170851
<i>stx1e</i>	Yes	Chromosomal -phage	Verotoxin 1e	KF926684
<i>stx2a</i>	Yes	Chromosomal -phage	Verotoxin 2a	X07865
<i>stx2b</i>	Yes	Chromosomal -phage	Verotoxin 2b	X65949
<i>stx2c</i>	Yes	Chromosomal -phage	Verotoxin 2c	M59432
<i>stx2d</i>	Yes	Chromosomal -phage	Verotoxin 2d	AF479828
<i>stx2e</i>	Yes	Chromosomal -phage	Verotoxin 2e	M21534
<i>stx2f</i>	Yes	Chromosomal -phage	Verotoxin 2f	AJ010730
<i>stx2g</i>	Yes	Chromosomal -phage	Verotoxin 2g	AY286000
<i>stx2h</i>	No	Chromosomal -phage	Verotoxin 2h	CP022279
<i>stx2i</i>	No	Chromosomal -phage	Verotoxin 2i	FN252457
<i>stx2k</i>	No	Chromosomal -phage	Verotoxin 2k	KC339670
<i>Stx2l</i>	No	Chromosomal -phage	Verotoxin 2l	AM904726
<i>aaiC</i>	Yes	EAEC pAA	AaiC, secreted protein	FN554766
<i>adfO</i>	No	O-Island 57	Adhesin	AE005174
<i>aggR</i>	Yes	EAEC Chromosome (Z32523)	Transcriptional activator	Z18751
<i>astA</i>	Yes	Plasmid and chromosome	Heat-stable enterotoxin	L11241 and HE603111
<i>bfpA</i>	No	pMAR2 plasmid (NC_011603)	Major structural subunit of bundle-forming pilus	AB247922 to AB247935
<i>cdt-V</i>	Yes	Chromosome (AJ508930)	Cytolethal distending toxin	JF461073
<i>chuAa</i>	No	Chromosome	Heme/hemoglobin receptor	AF280396
<i>cifD</i>	No	Chromosome	Deamidase	AY128535
<i>ckf</i>	No	O-Island 57	Putative killer protein	AE005174
<i>ecf1</i>	No	EHEC plasmid	Enzyme that enhances bacterial membrane structure	NC_007414
<i>ecf2</i>	No	EHEC plasmid	Enzyme that enhances bacterial membrane structure	NC_007414
<i>efa1</i>	No	O-Island 122	EHEC factor for adherence	AF159462
<i>eae</i>	Yes	LEE PI	Intimin	
<i>ehaA</i>	No	OI-15	Autotransporter of EHEC	AE005174
<i>ehxA</i>	No	EHEC plasmid (NC_007414)	Enterohemolysin	AF074613
<i>eibG</i>	No	aEHEC plasmid (NC_007365)	Immunoglobulin binding protein	AB255744
<i>ent/espL2</i>	No	O-Island 122	Microcolony formation and F-actin aggregation	AE005174
<i>epeA</i>	No	aEHEC plasmid	Serine protease autotransporter	AY258503.2; NC_007365
<i>espB</i>	No	LEE PI	LEE effector	Z21555

<i>espF</i>	No	LEE PI	LEE effector	AF116900
<i>espH</i>	No		Non-LEE-encoded effector	AB303061
<i>espJ</i>	No		Non-LEE-encoded effector	AB303061
<i>espK</i>	No	OI-50 (prophage CP-933N)	Non-LEE-encoded type III effector	AE005174
<i>espM1</i>	No	OI-71	Non-LEE-encoded type III effector	AE005174
<i>espN</i>	No	OI-50 (prophage CP-933N)	Non-LEE-encoded type III effector	AE005174
<i>espP</i>	No	EHEC plasmid (pO157)	Serine protease EspP	NC_002128
<i>espT</i>	No		RhoGEF mimic	
<i>espV</i>	No	OI-44	AvrA family effector	AE005174
<i>espZ</i>	No	Chromosome	–	DQ138078
<i>etpD</i>	No	pO157	Type-II effector	AF074613
<i>lha</i>	No	O-Island 43 and O-Island 48	Iron regulated adhesin;	AF126104
<i>iha_homologue</i>	No	O-Island 43 and O-Island 49	Iron regulated adhesin	AF126104
<i>irp2</i>	No	High pathogenicity island	Iron-repressible protein 2	CU928185
<i>katP</i>	No	EHEC plasmid (pO26)	Catalase peroxidase	GQ259888
<i>lpfAO113</i>	No	EAEC chromosome (CU928185)	Long polar fimbrial protein	AY057066
<i>lpfAO26</i>	No	EAEC chromosome (CU928185)	Major fimbrial subunit of LPFO26	AB161111
<i>mapR</i>	No		RhoGEF mimic	CAS11490
<i>nleA</i>	No	O-Island 71	Disruption of tight junctions and protein trafficking	AB303062
<i>nleB</i>	No	O-Island 122	Immunomodulation	AB303062
<i>nleB1</i>	No		Non-LEE encoded type III effector	FM180568
<i>nleB2</i>	No	O-Island 36	Non-LEE encoded type III effector	NC_013008
<i>nleC</i>	No	O-Island 36	Non-LEE encoded type III effector	AE005174
<i>nleD</i>	No	O-Island 36	Non-LEE encoded type III effector	AE005174
<i>nleE</i>	No	O-Island 122	Non-LEE encoded type III effector	AP010958
<i>nleF</i>	No	O-Island 71	Non-LEE encoded type III effector	AE005174
<i>nleG</i>	No	O-Island 71	Ubiquitin ligase	AB303062
<i>nleG2-1</i>	No	O-Island 71	Ubiquitin ligase	AP010953
<i>nleG2-3</i>	No	O-Island 57	Ubiquitin ligase	AP010953
<i>nleG5-2</i>	No	O-Island 57	Ubiquitin ligase	AE005174
<i>nleG6-2</i>	No	O-Island 57	Ubiquitin ligase	AE005174
<i>nleG9</i>	No	O-Island 71	Ubiquitin ligase	AP010953
<i>nleH1</i>	No	O-Island 36	Non-LEE encoded type III effector	AJA24806
<i>nleH2</i>	No	O-Island 71	Non-LEE encoded type III effector	AJA24806
<i>ompA</i>	No	Chromosome	Outer Membrane Protein II	V00307
<i>paa</i>	No	plasmid	porcine attaching-effacing associated protein	AY547306
<i>pagC</i>	No	OI-122	PagC-like membrane protein	AE005174
<i>saa</i>	No	pO113	STEC autoagglunating adhesin	NC_007365
<i>sab</i>	No	Plasmid	STEC autotransporter (AT) mediating biofilm formation	NC_007365
<i>subA</i>	No	pO113	Subtilase cytotoxin	NC_007414
<i>tia</i>	No	Chromosome	toxigenic invasion loci A	JQ994271

<i>tir</i>	Yes	LEE PI	translocated intimin receptor	AF013122
<i>toxB</i>	No	pO157	Homolog of efa1, adhesin	AF074613
<i>tspE4.C2</i>	No	Chromosome	Esterase-lipase protein	AF222188
<i>ureC</i>	No	OI-43 & OI-48	Urease-associated protein	NC002655
<i>ureD</i>	No	OI-43 and OI-48	Urease-associated protein UreD	AE005174
<i>wecA</i>	No	Chromosome	Polyisoprenyl-phosphate N-acetylhexosamine-1-phosphate transferase	

**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 (Catford et al., 2014). (Table 3 is related to the **Serotyping** section)

O-type	n	% of total of each O-type
O26	70	14.1
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
O4, O18, O21, O22, O38, O39, O40, O41, O49, O51, O52, O63, O68, O70, O75, O78, O79, O88, O98, O116, O136, O141, O171, O182, O183, O186, Inactive	1	0.2

Rough: isolate does not express lipopolysaccharide O chain.

Untypable: antibody reactions do not conform to serotyping scheme.



**Table 4.** Examples of foods internationally reported as sources of exposure to verotoxigenic *Escherichia coli*. Data and citations in Supplement 2. (Table 4 is related to the **Food and VTEC Exposure** section)

<b>Types</b>	<b>Reported Food Vehicles</b>
<b>Animal Origin</b>	
Meat	Beef, pork, mutton/lamb, bison, venison, chicken, kangaroo, turkey, pork pie, frankfurter, salami, deli meat, pepperoni, tartare, ham, kebab
Dairy	Cow's milk, Goat's milk, cheese, ice cream, cheese curds
Seafood	Salmon roe, tuna p <ac>o</ac> te, crab, salmon, lobster,
<b>Plant Origin</b>	
Fruit and Berries	Apple cider, tomato, cantaloupe, grapes, watermelon, fruit salad, strawberries, blueberries, pear
Herbs	Parsley, cilantro
Leafy Greens	Iceberg lettuce, romaine lettuce, spinach, kale, cabbage, arugula, rocket
Mushrooms	Unspecified
Nuts	Walnuts, Hazelnuts
Sprouts	Alfalfa, radish, fenugreek, clover, watercress, bean
Vegetables	Cucumber, celery, leeks, potatoes, green beans, onions, sugar peas
<b>Complex</b>	
Cold Prepared	Bean dip, guacamole, salsa, potato salad, pasta salad, coleslaw, bean salad, tuna salad, seafood salad, chocolate mousse, soy nut butter, mixed salads
Grains/Baked	Flour, brownie, cakes, cookie dough, wheat snack, pizza dough mix

**Table 5.** Incidents of foodborne verotoxigenic *Escherichia coli* illness with an identified food vehicle, internationally and in Canada, 1982 to 2018. Data and citations in Supplement 2. (Table 5 is related to the **Foods Associated with VTEC illness** section)

Total	Total				Canada			
	No. Incidents		No. Cases		No. Incidents		No. Cases	
	733		3,0786		189		3,003	
<b>Animal Origin</b>								
<b>Meat</b>	<b>377</b>	<b>51.3%</b>	<b>7,269</b>	<b>23.6%</b>	<b>142</b>	<b>75.1%</b>	<b>1,683</b>	<b>56.0%</b>
Beef	296	40.5%	4,877	15.8%	118	62.4%	1,044	34.8%
Bison	2	0.3%	22	0.1%	0	0.0%	0	0.0%
Chicken	6	0.8%	173	0.6%	2	1.1%	38	1.3%
Lamb/Mutton	6	0.8%	60	0.2%	0	0.0%	0	0.0%
Turkey	2	0.3%	38	0.1%	1	0.5%	36	1.2%
Venison	7	1.0%	72	0.2%	0	0.0%	0	0.0%
Kangaroo	1	0.5%	5		0	0.0%	0	0.0%
Pork	12	1.6%	328	1.1%	9	4.8%	288	9.6%
Unspecified	43	5.9%	1,694	5.5%	12	6.3%	277	9.2%
<b>Dairy</b>	<b>97</b>	<b>13.2%</b>	<b>1,385</b>	<b>4.5%</b>	<b>18</b>	<b>9.5%</b>	<b>229</b>	<b>7.6%</b>
Pasteurised/unspecified	25	3.4%	365	1.2%	2	1.1%	19	0.6%
Raw	72	9.8%	10,20	3.3%	16	8.5%	210	7.0%
<b>Seafood</b>	<b>7</b>	<b>1.0%</b>	<b>96</b>	<b>0.3%</b>	<b>1</b>	<b>0.5%</b>	<b>3</b>	<b>0.1%</b>
<b>Plant Origin</b>	<b>137</b>	<b>18.7%</b>	<b>17,694</b>	<b>57.5%</b>	<b>13</b>	<b>6.9%</b>	<b>661</b>	<b>22.0%</b>
Fruit and Berries	30	4.1%	1,551	5.0%	4	2.1%	166	5.5%
Leafy Greens	71	9.7%	2,675	8.7%	5	2.6%	214	7.1%
Nuts	3	0.4%	30	0.1%	2	1.1%	22	0.7%
Sprouts	18	2.5%	1,2450	40.4%	1	0.5%	24	0.8%
Vegetables	11	1.5%	788	2.6%	1	0.5%	235	7.8%
Herbs	3	0.4%	150	0.5%	0	0.0%	0	0.0%
Mushrooms	1	0.1%	50	0.2%	0	0.0%	0	0.0%
<b>Complex</b>	<b>116</b>	<b>15.8%</b>	<b>4,342</b>	<b>14.1%</b>	<b>15</b>	<b>7.9%</b>	<b>427</b>	<b>14.2%</b>
Cold Prepared	47	6.4%	1,866	6.1%	3	1.6%	223	7.4%
Grains/Baked	10	1.4%	268	0.9%	3	1.6%	37	1.2%
Multiple	59	8.0%	2,208	7.2%	9	4.8%	167	5.6%

**Table 6.** Prevalence of verotoxigenic *Escherichia coli* in FoodNet retail samples, irrigation water and feedlot beef manure samples, 2014-2017. (Table 6 is related to the **Foods Associated with VTEC illness, FoodNet Canada** and **Raw Ground Beef, Pork and Veal and Precursor Materials** sections)

Sampling Analytical Unit		Retail Ground Beef	Retail Ground Pork n=1/250 g 25 g	Retail Veal	Irrigation Water n=1/1000 mL 150 mL	Feedlot Cattle Manure n=1/110 g 1
<b>2014</b>	n	296	23	ND	149	ND
	Positive	5 (1.7%)	1 (4.4%)	ND	41 (27.5%)	ND
<b>2015</b>	n	387	75	ND	188	ND
	Positive	9 (2.3%)	5 (6.7%)	ND	60 (31.9%)	ND
<b>2016</b>	n	393	ND	ND	142	78
	Positive	5 (1.3%)	ND	ND	41 (28.9%)	8 (10.3%)
<b>2017</b>	n	382	ND	334	116	76
	Positive	10 (2.6%)	ND	21 (6.3%)	38 (32.8%)	13 (17.1%)
<b>Total</b>	n	1458	98	334	595	154
	Positive	29 (2.0%)	6 (6.1%)	21 (6.3%)	180 (30.3%)	21 (13.64%)

ND: Not done

**Table 7.** Summary of food monitoring studies for verotoxigenic *Escherichia coli* in Canada from April 1, 2013 to March 31, 2018. (Table 7 is related to the **Foods Associated with VTEC illness, Targeted Surveys Program, Raw Ground Beef, Pork and Veal and Precursor Materials, Ready-to-Eat Meats, Raw Milk Cheeses, Fresh Produce and Other Plant-Based Foods** sections)

Food Type	Sampling Program	Lot Sampling	Analytical Unit	Origin	Target Serotype(s)	Tested	Positive	Serotypes
Raw ground pork, beef and veal	NMMP	n=5/200 g	325 g <sup>e</sup>	Domestic	O157:H7/NM	3,273	3	O157:H7/NM
	NMMP	n=5/200 g	325 g <sup>e</sup>	Imported	O157:H7/NM	48	0	
Raw ground beef precursor	NMMP	N60 <sup>a</sup>	325 g composite	Domestic	O157:H7/NM	3,834	5	O157:H7/NM
	NMMP	N60 <sup>a</sup>	325 g composite	Imported	O157:H7/NM	165	0	
Ready-to-eat meats	NMMP	n=5/250 g	325 g <sup>e</sup>	Domestic	O157:H7/NM	22	0	
	NMMP	n=5/250 g	325 g <sup>e</sup>	Imported	O157:H7/NM	15	0	
Raw milk cheeses	NMMP	n=5/200 g to 1 Kg	125 g <sup>d</sup>	Domestic	O157:H7/NM	247	0	
	NMMP	n=5/200 g to 1 Kg	125 g <sup>d</sup>	Imported	O157:H7/NM	550	0	
Fresh and fresh-cut ready-to-eat fruits and vegetables	NMMP and FSO	n=1 or 5 <sup>b</sup> /150-250 g <sup>c</sup>	25 g for n = 1 or 125 g <sup>d</sup> for n=5	Domestic	O157:H7/NM	2,617	0	
	NMMP	n=5/150 g <sup>b</sup>	125 g <sup>d</sup>	Domestic	All	66	0	
	NMMP and FSO	n=1 or 5 <sup>b</sup> /150-250 g <sup>c</sup>	25 g for n = 1 or 125 g <sup>d</sup> for n=5	Imported	All	4,882	0	
	NMMP	n=5/150 g	125 g <sup>d</sup>	Imported	All	187	0	
	Targeted Surveys	n=1/250 g	25 g	Both	O157:H7/NM	28,715	0	
Nuts and nut butters	Targeted Surveys	n=1/250 g	25 g	Both	All	1,251	6	all non-O157
	Targeted Surveys (2013-2014)	n=1/250 g	25 g	Both	O157:H7/NM	3,972	0	
Dried sprouted seeds	Targeted Surveys (1 year)	n=1/250 g	25 g	Both	O157:H7/NM	322	0	
	Targeted Surveys (Multi year)	n=1/250 g	25 g	Both	All	1,028	4	all non-O157
unpasteurized juices and ciders	Targeted Surveys (2016-2017)	n=1/250 ml	25 g	Both	O157:H7/NM	1,133	0	

a. N60: thin slices of approximately 50 cm<sup>2</sup> are collected from the surface of 60 pieces of precursor materials.

b. NMMP and FSO samples collected by CFIA inspectors at domestic establishments and importers consisted of 5 subunits. FSO and Targeted Survey samples collected at retail consisted of 1 subunit.

- c. Institutional-sized bags of pre-products, collected by CFIA inspectors, that were destined for restaurants, hospitals or institutions, could be less than five (5) units as long as the total weight is at least 1000 g. For whole large fruits, such as cantaloupes, melons & papayas, a single fruit is sampled for each test.
- d. 5 x 25 g composite
- e. 5 x 65 g composite

**Table 8.** Incidents of foodborne verotoxigenic *Escherichia coli* reported internationally, fifteen largest outbreaks by number of cases. Data and citations in Supplement 2. (Table 8 is related to the **Food Preparation Practices Associated with VTEC Illness** section)

Location	Year	Serotype	Cases	Deaths	Vehicle
Japan	1996	O157:H7	8,355	NA	Radish sprouts
Germany	2011	O104:H4	3,816	54	Fenugreek sprouts
USA	2000	O157:H7	736	1	Watermelon
UK	1996	O157:H7	512	17	Various cooked meats
USA	1992	O157:H7	477	3	Hamburger
USA	2008	O111:NM	341	1	Restaurant meals
USA	1999	O157:H7	321	0	Beef
Japan	2007	O157:H7	314	NA	Boxed meals
Japan	2011	O157:H7	304	1	Rice cakes
UK	2005	O157	275	1	Meat, cross contamination
UK	2010	O157	252	1	Raw leeks and potatoes
USA	2006	O157:H7	238	5	Spinach
Finland	2016	ONT:H11	237	0	Rocket salad
Canada	2008	O157:H7	235	0	Onion
Japan	1996	O157:H7	215	NA	Seafood salad

ONT: O group not typable

**Table 9.** Incidents of foodborne verotoxigenic *Escherichia coli* reported in Canada, fifteen largest outbreaks by number of cases. Data and citations in Supplement 2. (Table 9 is related to the **Food Preparation Practices Associated with VTEC Illness** section)

Province	Year	Serotype	Cases	Deaths	Vehicle
Ontario	2008	O157:H7	235	0	Onion
Nova Scotia	1998	O157	182	0	Salad
Quebec	2000	O157:H7	176	0	Ground Beef
Ontario	2008	O157:H7	148	0	Romaine lettuce
Canada	1999	O157:H7	143	0	Sausage
Alberta	2014	O157:H7	119	0	Pork
Saskatchewan	2001	O157:H7	79	0	Pork
Ontario	1985	O157:H7	70	17	Ham Sandwiches
Canada/USA	1996	O157:H7	70	1	Apple juice, unpasteurized
Ontario	2003	O157:H7	61	0	Haggis
Manitoba	2006	O157	57	0	Hamburger
Alberta	2004	O157:H7	51	0	Beef donair
Ontario	1986	O157:H7	47	0	Raw milk
Canada	2007	O157:H7	46	1	Ground Beef

**Table 10.** Levels of verotoxigenic *Escherichia coli* reported in outbreak associated foods. (Table 10 is related to the **Levels of VTEC in Outbreak-Associated Foods** section)

<b>Food</b>	<b>Serotype</b>	<b>Level</b>	<b>Citation</b>
Fermented Sausage	O157:H7	0.4 CFU/g	Tilden et al., 1996
Beef patties	O157:H7	<13.7 to 675 CFU/45g	Tuttle et al., 1999
Raw milk cheese	O157:H7	5 to 10 CFU/g	Strachan et al., 2001
Seafood sauce	O157:H7	0.11 CFU/g	Teunis et al., 2004
Beef patties	O157:H7	1.45 MPN/g	Hara-Kudo and Takatori, 2011
Beef	O157:H7	23 MPN/g	Hara-Kudo and Takatori, 2011
Raw milk cheese	O157:H7	0.37 to 0.95 MPN/100g	Gill and Oudit 2015
Beef patties	O157:H7	2.2 MPN/100g	Gill and Huszczyński, 2016
Minced meat cutlets (beef, pork, onions, and eggs)	O157:H7	2.3 to 110 MPN/g	Furukawa et al. 2018
Fermented sausage	O111:H-	0.1 CFU/g	Paton et al., 1996
Ice cream	O26:H11	0.03 MPN/g	Buvens et al., 2011
	O145:H28	2.4 MPN/g	
Wheat flour	O121:H19	0.17 to 0.43 MPN/100g	Gill et al., 2019a

CFU: colony forming units

MPN: most probable number



**Table 11.** Serotypes of verotoxigenic *Escherichia coli* isolated from FoodNet retail samples, irrigation water and feedlot beef manure samples, 2014-2017. (Table 11 is related to the **FoodNetCanada** section)

<b>Retail Ground Beef</b> All sites	<b>Retail Ground Pork</b> All sites	<b>Retail Veal</b> All sites	<b>Irrigation Water</b> SS2-a, SS3-a	<b>Feedlot Beef Manure</b> SS3-a
O?:H21, O5:NM, O6:H34, O25, O26:H11, O34:H32, O39:H21, O41, O46:H38, O76:H19, O76:NM, O91:H21, O103:H2, O113:H21, O117:H2, O136:H12, O141AC:H2, O146:H8, O157:H7, O168:H8, O171:H2, O177:NM, untyped	O2:NM, O8, O8:H19, O100:NM, O103:H2, O121:H10, O145:NM, O155:H20, O157:H16, O157:H7, O163:H19, O163:NM, untyped	O?:H5, O2:H29, O8:H19, O55:H12, O91:NM, O109:H5, O111:NM, O113:NM, O118:H16, O132:NM, O157:H7, O160:H12, O174:H21, O185:H7	O2, O3, O4, O5, O6, O7, O8, O11, O22, O26, O34, O36, O39, O41, O43, O45, O51, O54, O55, O63, O75, O76, O83, O84, O88, O91, O98, O103, O106, O109, O111, O112, O113, O114, O115, O116, O121, O126, O128, O130, O132, O136, O145, O152, O153, O157, O159, O163, O165, O166, O168, O172, O174, O177, O178, O179, O181, O182, O183, O185, O187, O188, Untyped	O2, O76, O88, O104, O109, O132, O145, O157, O163, O168, O171

**Table 12.** Government of Canada documents providing guidance on verotoxigenic *Escherichia coli* in foods. (Table 12 is related to the **Meat** and **Fresh Produce** sections)

Title	Link
Health Canada’s Guidance Document on <i>Escherichia coli</i> O157:H7 and <i>E. coli</i> O157:NM in Raw Beef	<a href="https://www.canada.ca/en/health-canada/services/food-nutrition/legislation-guidelines/guidance-documents/guidance-document-coli-0157-coli-0157-beef-2014.html">https://www.canada.ca/en/health-canada/services/food-nutrition/legislation-guidelines/guidance-documents/guidance-document-coli-0157-coli-0157-beef-2014.html</a>
Interim guidelines for the control of verotoxigenic <i>Escherichia coli</i> including <i>E. coli</i> O157:H7 in ready to eat fermented sausages containing beef or a beef product as an ingredient	<a href="https://www.canada.ca/en/health-canada/services/food-nutrition/legislation-guidelines/guidance-documents/interim-guidelines-control-verotoxinogenic-escherichia-coli-including-colio157-fermented-sausages-beef-product-ingredient.html">https://www.canada.ca/en/health-canada/services/food-nutrition/legislation-guidelines/guidance-documents/interim-guidelines-control-verotoxinogenic-escherichia-coli-including-colio157-fermented-sausages-beef-product-ingredient.html</a>
Guidance on Mandatory Labelling for Mechanically Tenderized Beef	<a href="https://www.canada.ca/en/health-canada/services/food-nutrition/legislation-guidelines/guidance-documents/guidance-mandatory-labelling-mechanically-tenderized-beef.html">https://www.canada.ca/en/health-canada/services/food-nutrition/legislation-guidelines/guidance-documents/guidance-mandatory-labelling-mechanically-tenderized-beef.html</a>
Management of the Risks Related to the Consumption of Donairs and Similar Products	<a href="https://www.canada.ca/en/health-canada/services/food-nutrition/legislation-guidelines/guidance-documents/management-risks-related-consumption-donairs-similar-products-gyros-kebabs-chawarmas-shawarmas-2008.html">https://www.canada.ca/en/health-canada/services/food-nutrition/legislation-guidelines/guidance-documents/management-risks-related-consumption-donairs-similar-products-gyros-kebabs-chawarmas-shawarmas-2008.html</a>
Food and Drug Regulations- FDR- (C.R.C., c. 870), B.08.002.2 (1)	<a href="https://laws-lois.justice.gc.ca/eng/regulations/C.R.C.,_c._870/page-40.html#h-71">https://laws-lois.justice.gc.ca/eng/regulations/C.R.C.,_c._870/page-40.html#h-71</a>
Managing Health Risks Associated with Unpasteurized Fruit Juice Cider Products	<a href="https://www.canada.ca/en/health-canada/services/food-nutrition/legislation-guidelines/policies/managing-health-risk-associated-consumption-unpasteurized-fruit-juice-cider-products.html">https://www.canada.ca/en/health-canada/services/food-nutrition/legislation-guidelines/policies/managing-health-risk-associated-consumption-unpasteurized-fruit-juice-cider-products.html</a>
Code of Practice for the Production and Distribution of Unpasteurized Apple and Other Fruit Juice/Cider in Canada	<a href="http://www.inspection.gc.ca/food/processed-products/manuals/code-of-practice/eng/1340636187830/1340637184931">http://www.inspection.gc.ca/food/processed-products/manuals/code-of-practice/eng/1340636187830/1340637184931</a>
Policy on Managing Health Risk Associated with the Consumption of Sprouted Seeds and Beans	<a href="https://www.canada.ca/en/health-canada/services/food-nutrition/legislation-guidelines/policies/policy-managing-health-risk-associated-consumption-sprouted-seeds-beans.html">https://www.canada.ca/en/health-canada/services/food-nutrition/legislation-guidelines/policies/policy-managing-health-risk-associated-consumption-sprouted-seeds-beans.html</a>