Non-O157 verotoxigenic *Escherichia coli* and beef: A Canadian perspective

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Abstract

Verotoxigenic *Escherichia coli* (VTEC) are important foodborne pathogens in Canada. VTEC of the O157:H7 serogroup have been the focus of regulatory action and surveillance in both Canada and the USA, due to their role in a number of high profile outbreaks. However, there is increasing evidence that other VTEC serogroups cause a substantial proportion of human illness. This issue is of particular importance to the cattle industry due to the role of beef as a vehicle for VTEC transmission. In this review, the evidence for non-O157 VTEC as cause of human illness in Canada and the potential for Canadian beef and cattle to serve as a source of VTEC are presented. In addition, the available strategies for the control of VTEC in cattle and beef are discussed.

Résumé

Au Canada les Escherichia coli *vérotoxinogènes (VTEC) sont d'importants agents pathogènes d'origine alimentaire. Les VTEC du sérogroupe O157:H7 ont été l'objet d'action réglementaire et de surveillance au Canada et aux États-Unis, étant donné leur rôle dans plusieurs épisodes à haute visibilité. Toutefois, il y a de plus en plus d'évidences que d'autres sérogroupes de VTEC causent une proportion importante des cas de maladie humaine. Cet enjeu revêt une importance particulière pour l'industrie du bétail étant donné le rôle joué par le bœuf en tant que véhicule pour la transmission de VTEC. Dans la présente revue, on présente les évidences relatives au rôle joué par les VTEC non-O157 en tant que cause de maladie humaine au Canada et le potentiel pour le bœuf et les vaches d'origine canadienne d'être la source de VTEC. De plus, les stratégies disponibles pour la maîtrise des STEC chez le bœuf et la vaches sont discutées.*

(Traduit par Docteur Serge Messier)

Introduction

Verotoxigenic *Escherichia coli* are the pathogenic *E. coli* of greatest public health concern in Canada, and were first recognized as such in 1982 following outbreaks involving the O157:H7 serogroup in the USA (1). Since then, VTEC have been identified as significant causes of sporadic and outbreak cases of foodborne disease throughout the world. VTEC illnesses commonly conclude with a self-limiting episode of bloody diarrhea, [hemorrhagic colitis (HC)], but 5% to 15% of cases can progress to life-threatening hemolytic-uremic syndrome (HUS) (2,3). Death may result in up to 12% of HUS cases, and 25% of survivors are left with long-term health problems (4).

Because of their role in a number of high profile outbreaks, *E. coli* O157:H7 and its nonmotile mutants (O157:NM or O157:H-) have been the focus of regulatory action and surveillance in both Canada and the USA. These "typical" *E. coli* O157:H7 are non-sorbitol fermenting and negative for the enzyme β-D-glucuronidase, characteristics that are commonly used for their isolation and identification. The importance accorded to the *E. coli* O157:H7 serogroup is exemplified by it being designated a food adulterant by the United States Department of Agriculture-Food Safety and Inspection Service (USDA-FSIS) in 1994 and the subsequent development of a program to test for its presence in beef. The Canadian regulations on *E. coli* O157:H7 are set out in Health Canada Guidelines 10 and 12, which stipulate procedures for the handling of *E. coli* O157:H7 positive raw ground beef (5) and for the preparation of fermented meat products containing beef (6). VTEC infection has been classified as a notifiable disease in Canada since 1990, and it is subject to national reporting by the Public Health Agency of Canada. In the USA, *E. coli* O157:H7 infection has been classified as a nationally notifiable disease since 1995, with infections by other VTEC serogroups added in 2000. The regulatory and surveillance focus on *E. coli* O157:H7 was aided by the relative ease with which this serogroup can be detected compared with other VTEC, which in turn reinforced a common assumption that non-O157 VTEC disease occurred infrequently in both Canada and the USA.

This picture of the dominance of the O157:H7 serogroup as a cause of VTEC illness in North America has been increasingly challenged by the findings of studies indicating that non-O157 may be the cause of 30% to 50% of VTEC illness (7–11). Reports from other countries, including those that export beef to Canada and the USA, indicated that non-O157 VTEC are of primary importance elsewhere (12,13). Consequently, USA authorities are taking an increasing interest in non-O157 VTEC. In 2007, the USDA issued a report on non-O157 VTEC (14) and in 2008 USDA-FSIS officials announced the intention to begin testing foods for the presence of 5 VTEC serogroups

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Table I. Seropathotype scheme for Verotoxigenic *Escherichia coli* as proposed by Karmali et al (20)

Seropathotype	Relative incidence	Outbreaks	Cause of HC or HUS	Serogroup
A	High	Common	Yes	O157:H7, O157:NM
B	Moderate	Uncommon	Yes	O26:H11, O103:H2, O111:NM, O121:H19, O145:NM
C	Low	Rare	Yes	O91:H21, O104:H21, O113:H21, others
D	Low	Rare	No	Multiple
E	Nonhuman	NA	NA	Multiple

HC — hemmorrhagic colitis.
HUS — hemolytic uremic syndrome.
NA — not applicable.
Adapted from reference 20.

Table II. Factors used to identify typical *Escherichia coli* O157 and their presence in non-O157 VTEC and other *E. coli* types, including pathogens and commensal isolates

	Presence in isolates		
Factor	Typical O157:H7	Non-O157 VTEC	Other *E. coli*
Virulence factors			
Verotoxin	Yes	Yes	No
Locus of enterocyte effacement	Yes	Variable	Variable
EHEC-enterohaemolysin	Yes	Variable	Variable
Phenotypic traits			
Sorbitol fermentation	No	Variable	Variable
β-D-glucuronidase	No	Variable	Variable
O157 antigen	Yes	No	Variable
H7 antigen	Variable	Variable	Variable

Yes — present in isolates.
Variable — may or may not be present in isolates.
No — not present in isolates.

(O26, O45, O103, O111, and O145). The testing would obtain data to aid the decision on whether or not these serogroups as well as O157:H7 should be treated as adulterants (15). Although an official position on non-O157 VTEC has as yet to be adopted in Canada, the high degree of integration between the USA and Canadian beef industries and the importance of the USA as an export market for Canadian beef ensures that any regulatory changes in the USA will necessarily affect the Canadian beef industry. Therefore, the current state of knowledge of non-O157 VTEC as causes of human illness and their occurrence in Canadian and USA beef and beef production operations is considered in this review.

Terminology

The terms verotoxin and VTEC are used herein to describe the toxins and the *E. coli* strains which express them. In the scientific literature, the terms verocytotoxin and Shiga toxin are also used for verotoxin and thus the 3 terms can be regarded as being interchangeable. The term shigatoxigenic (STEC) is equivalent to VTEC, being defined as *E. coli* that possess the verotoxin virulence factor. The term enterohemorrhagic *E. coli* (EHEC) was originally proposed by Levine (16) to designate a subgroup of VTEC responsible for serious human illness; EHEC being defined as *E. coli* that cause HC and HUS, and that possess the virulence factors of verotoxin expression, the locus of enterocyte effacement (LEE) and a 60 MDa EHEC plasmid. However, EHEC is used confusingly in the literature to designate VTEC isolates associated with HC and HUS, whether or not they possess the LEE and the EHEC plasmid.

VTEC serotype

Serological typing of *E. coli* was originally introduced to distinguish between isolates based on the binding of antibodies to antigenic cell surface structures. The current serotyping scheme for *E. coli* was introduced by Kauffmann in 1947 (17). The serotypes of *E. coli* isolates are defined by 3 antigen types, O, H, and K. The O-antigen is the polysaccharide portion of the outer membrane lipopolysaccharide and the H-antigen is the protein flagellin, which comprises the filament of the bacterial flagellium. The K-antigens consist of diverse acidic capsular polysaccharides (18). Isolates are often designated by O- and H-antigens only. Thus, the designation O157:H7 indicates the 157th O-antigen and the 7th H-antigen. To date, approximately 180 O and 60 H antigens have been identified for *E. coli* (19).

Historically, serotyping has been an important tool for the identification of potentially pathogenic *E. coli* including VTEC, because in most cases there are no physiological characteristics which allow pathogenic strains to be distinguished from commensal *E. coli.* In the absence of identified virulence factors and methods for their

detection, serotyping was a convenient means for identifying presumptive VTEC, as some serogroups have a higher association with pathogenicity than others. Karmali et al, (20) proposed, on the basis of American and Canadian data, that VTEC can be divided into 5 seropathotypes (A to E) that reflect their relative epidemiological importance (Table I). The seropathotype division is based on each serogroup's relative incidence as a cause of illness, its involvement in outbreaks, and its association with HC and HUS. Unfortunately, the only virulence factor shared by all known VTEC isolates from HC and HUS patients is verotoxin secretion. The genetic traits which result in isolates of some serogroups, such as O157:H7, being more commonly associated than others with outbreaks and serious illness in Canada and the USA remain obscure.

When considering data on the serogroups of *E. coli* isolates it is important to appreciate that not only are the O- and H-antigens not intrinsic virulence factors but, in addition, they are not genetically linked to any known virulence factors. Thus, identification of an isolate as belonging to a specific serogroup associated with pathogenicity does not establish that it is in fact a pathogen, but only that the probability of it being a pathogen is greater than that for some other serogroups. This point is illustrated by reports of verotoxin negative *E. coli* O157:H7 (21,22). Conversely, no serogroup can be assumed to be non-pathogenic. A survey of VTEC O serotypes reported worldwide found that in 3760 incidences of VTEC isolation, 162 of the 180 O serotypes were represented (13).

Virulence factors

Verotoxin

The defining characteristic of VTEC is the virulence factor verotoxin. Although various other virulence factors have been identified in VTEC isolates, the possession of a gene or genes for, and expression of one or more verotoxins remains the sole commonality of VTEC isolates (23) (Table II).

The first report of verotoxins was by Konowalchuk et al (24), who named them for their toxicity to cultured vero cells. Verotoxins consist of 2 protein subunits, A and B. The A subunit of about 33 kDa is enzymatically active and enters the target eukaryotic cell where it cleaves a single adenine nucleotide from the 28S ribosomal subunit, resulting in inhibition of tRNA binding and termination of protein synthesis (25). The B subunit has a size of 7.5 kDa and forms a pentamer, which binds to a specific cell receptor to provoke internalization of the toxin by the target cell (25). The name Shiga toxin (Stx) is also used because of the extensive DNA sequence homology of verotoxins genes with the Stx of *Shigella dysenteriae*.

Currently, 6 verotoxin variants, VT1, VT2, VT2c, VT2d, VT2e, and VT2f are commonly recognized, based on differences in homology with Shiga toxin and in the targeted receptor (18). The genes for both the A and B subunits of VT1 have > 99% nucleotide sequence identity with the Stx of *S. dysenteriae*, and the 2 toxins are immunologically and functionally indistinguishable (23). The gene for the A subunit of VT2 has 57% and the gene for the B subunit 60% nucleotide identity with Stx. The genes for the remaining VT2 variants (VT2c, VT2d, VT2e, and VT2f) have between 95% and 78% nucleotide identity with the A subunit of VT2 and 95% to 70% nucleotide identity with the B subunit (23). The variation in B subunit identity can affect the target specificity of the toxin. Most VT toxins interact with the receptor globotriaosylceramide (Gb3) a membrane glycolipid, but VT2e and VT2f are specific for the receptor globotetraosylceramide (Gb4) (23). The genes for all verotoxins are encoded on the chromosome within integrated lambda phages or flanked by phage sequences. This indicates their origin in phage-mediated gene transfer events, which provide a mechanism for the transfer of verotoxin genes between unrelated strains of *E. coli.* (26,27).

There is considerable variation in the cytotoxicity of the verotoxin variants in cell culture assays, with purified VT2 being reported to have a toxicity 1000× greater for human renal microvascular endothelial cells than VT1 (28). Isolates carrying VT2 and VT2c have been reported to have higher probabilities of association with serious VTEC illness than isolates of other verotoxin type (10,29). However, isolates expressing other verotoxins can not be assumed to be less pathogenic, as isolates positive for VT1 only have been recovered from patients suffering from HC and HUS (10,29).

Additional virulence factors

A large number of additional virulence factors have been identified in specific VTEC isolates, or proposed on the basis of sequence homology with virulence factors found in other organisms. Two additional virulence factors have been shown to correlate to patients suffering from HC and HUS. These are the LEE pathogenicity island and EHEC-enterohemolysin.

The LEE consists of up to 50 genes grouped in a 35–40 kb pathogenicity island on the chromosome. These genes encode for proteins involved in the formation of attaching/effacing (AE) lesions. AE lesions result from the formation of a tight, protein-mediated connection of an *E. coli* cell to a host epithelial cell, followed by modification of the host cytoskeleton. This causes the *E. coli* cell to be raised on a pedestal formed by the epithelial cell (30). Genes identified in the LEE include those that code for a type III protein secretion system, the intimin adhesin *(eae)*, the translocated intimin adhesin receptor *(tir)*, and several additional effector proteins injected into the host cell by the type III secretion system (23). Polymerase chain reaction (PCR) tests to identify the presence of the LEE typically use probes for the intimin gene, *eae.*

EHEC-enterohemolysin (EHEC-HlyA or EhxA) was initially identified as the protein responsible for hemolytic activity of *E. coli* O157:H7 on washed sheep blood agar (31). The EHEC-enterohemolysin gene, *ehxA* is encoded on a 60 MDa plasmid with the genes for 3 accessory proteins, B, C, and D that are involved in its expression and secretion (23). EHEC-enterohemolysin is a member of the RTX toxin family and the EhxA protein forms pores in the cytoplasmic membranes of target cells (23). Whether or not EhxA has any role in human or animal illness is unknown.

Although LEE and EHEC-enterohemolysin are strongly associated with VTEC isolates that cause HC and HUS, their presence or absence in *E. coli* does not seem to determine the ability of isolates to cause human disease. A study of 56 clinical isolates of non-O157 VTEC collected in Finland from 1990 to 2000, found that 70% were positive for eae and 75% for ehxA; however, 20% of the isolates carried neither gene (32). Similarly, a USA study of 940 clinical VTEC isolates collected from 1983 to 2002, found that 84% and 86% of the isolates were positive for *eae* and *ehxA*, respectively (10).

A number of putative VTEC virulence factors have been proposed, usually on the basis of sequence homology. These include a type II secretion system, serine proteases, urease, subtilase cytotoxin, cytolethal distending toxin, fimbriae and nonfimbrial adhesins (23,33,34). However, it is uncertain if these proteins play any role in pathogenicity.

Bettelheim (13) proposed that the failure to identify virulence factors other than the verotoxin present in all VTEC isolates could be due to co-infection of patients with multiple VTEC strains, with the virulence factors required for serious illness being supplied by several strains. Since most bacteriological investigations of illness end upon the isolation of a single identifiable pathogen, which could cause the observed symptoms, it is likely that co-infections are rarely detected.

Non-O157 VTEC illness in Canada and the USA

Although certain serogroups of *E. coli* have higher rates of association with VTEC illness than others, the important serogroups and their incidences vary greatly among geographic regions (12,33). In Canada and the USA, O157:H7 appears to be the single most prevalent VTEC serogroup, but the available evidence indicates that other serogroups contribute significantly to human illness.

Neither the Public Health Agency of Canada nor any of the Canadian provincial health organizations systematically test for non-O157 VTEC. Systematic testing of clinical samples for VTEC regardless of serogroup is complicated by the absence of any physiological characteristics, other than verotoxin, that can be used to distinguish them from commensal *E. coli*. Unlike most VTEC and commensal *E. coli*, typical *E. coli* O157:H7 isolates are unable to ferment sorbitol and do not express the enzyme β-D-glucuronidase.

The Public Health Agency of Canada reported the incidence of illness in the human population caused by pathogenic *E. coli* in Canada for 2004 and 2006 as 3.4/100 000 and 2.9/100 000, respectively (35,36). However, these reports include data only on O157:H7 VTEC, for though VTEC disease is notifiable in Canada, very few public health laboratories conduct tests for non-O157 VTEC. A review of other studies provides evidence that relatively few cases of non-O157 VTEC illness are reported only because of the absence of systematic testing for these organisms.

Surveillance for verotoxin in the of stool specimens from diarrheal patients, conducted in Calgary for 10 wk during 1990, detected verotoxin in 2.1% of 3577 samples (37). However, VTEC O157:H7 were isolated from only 0.6% of the samples, which suggests that a substantial portion of the cases of VTEC illness were due to non-O157 VTEC. Two studies have been conducted in Manitoba to systematically detect and isolate O157:H7 and non-O157 VTEC from patients suffering from diarrheal illness or HUS (9,38). In the first study, 968 stool samples were screened for verotoxin, and 31 were positive. Isolates were recovered from 23 of the positive samples, with 12 isolates being O157:H7 and 11 being non-O157 (38). In a 2nd study, using PCR screening of samples for verotoxin genes and conducted over 12 mo in 2003–2004, 30 VTEC isolates were recovered, and 19 (63%) were identified as non-O157 (9). These results indicate that serogroups other than O157:H7 are responsible for a significant proportion of VTEC illness in Canada.

The suggestion that the apparently low prevalence of non-O157 VTEC in Canada is due to under reporting is supported by recent studies in the USA. Surveillance of samples from patients for non-O157 VTEC, in a number of USA states, has found much higher than expected levels of non-O157 VTEC illness. In Idaho, 55% of VTEC illness was attributed to non-O157 strains (39). Studies conducted in Virginia, Nebraska, and Connecticut found that 31%, 50%, and 51% of VTEC illness, respectively, was associated with non-O157 VTEC (7,8,40). In response to reports of high non-O157 VTEC prevalence in the United States, the Centers for Disease Control has recently begun surveillance for non-O157 VTEC through its FoodNet program. In 2007 the incidence of laboratory confirmed cases of O157:H7 VTEC illness reported in the FoodNet population of 45.5 million persons in 10 states was 1.20 per 100 000, and the incidence of non-O157 VTEC illness was 0.57 per 100 000 (11).

Thus, it appears that VTEC serogroups other than O157:H7 maybe responsible for up to 50% of VTEC illness in both Canada and the USA. The uncertainty about the incidence of non-O157 VTEC in Canada and the USA is due to a lack of routine testing for these organisms. This is in part a consequence of the relative ease with which samples can be screened for typical *E. coli* O157:H7, which possess 2 unique physiological characteristics (non-sorbitol fermenting and β-D-glucuronidase negative) that are not shared by other VTEC.

The announcement by the USDA-FSIS of its intention to expand testing of beef to include 5 *E. coli* serogroups (O26, O45, O103, O111, O145) in addition to O157:H7 reflects the involvement of these 5 serogroups in VTEC illness in the USA. These 5 serogroups accounted for 70% of the cases of non-O157 VTEC illness reported in the USA from 1983 to 2002. Rates for the individual serogroups were 22%, 16%, 12%, 8%, 7%, and 5% for O26, O111, O103, O121, O45, and O145, respectively. However, other serogroups were responsible for 30% of VTEC illness during the same period (10).

Thirteen outbreaks caused by non-O157 VTEC of serogroups O26, O45, O51, O103, O104, O111, and O121 were reported in the USA from 1990 to 2006 (10,14). None of these outbreaks was linked to consumption of beef products, though 2 were linked to contact with calves. The potential for beef to serve as a source of non-O157 VTEC can not be discounted, given that most cases of VTEC illness are sporadic, and that the sources of such infections are not usually investigated. The involvement of beef in non-O157 VTEC illness was indicated in an epidemiological investigation of sporadic cases of VTEC illness in Argentina. In this study, consumption of under cooked beef was identified as a major risk factor for childhood illness associated with non-O157 VTEC (41).

Non-O157 VTEC in Canadian and American cattle

It is well-established that cattle are a reservoir for both O157:H7 and non-O157 VTEC. Verotoxigenic *E. coli* can cause mild diarrheal illness in calves, but shedding by adult cattle is not associated with illness (42,43). Shedding of VTEC by adult cattle is episodic and generally found to be greater during the warmer months of the year (44).

A survey of reports from around the world on the prevalence of O157:H7 VTEC in beef cattle, as indicated by examination of fecal samples from cattle presented for slaughter, was that the reported rates ranged from 0.2% to 27.8% (45). The rates for non-O157 VTEC

prevalence ranged from 2.1% to 70.1% (45). Results obtained in studies conducted in the USA and Canada were comparable to those obtained in other regions, with 0.3% to 27.8% of samples being positive for O157:H7 VTEC in 13 studies, and 3.6% to 19.4% of samples being positive for non-O157 VTEC in 4 studies (45). The wide ranges of the reported prevalence is very likely a consequence of not only differences in climate, ecology, and farming practice in different regions, but also of variation in the sampling and testing protocols used in different studies. However, it is clear that non-O157 VTEC are carried by substantial portions of both Canadian and American cattle.

As with beef and clinical samples, the prevalence of VTEC is higher when testing of cattle feces, hides, and carcasses is not limited to the O157:H7 serogroup. In testing of fecal swabs from 1000 Prince Edward Island beef cattle, VTEC isolates were recovered from 4% of animals (46). Screening of feces from 1247 cattle at an Alberta abattoir found *E. coli* O157:H7 in only 7.5% of samples, whereas 42.6% of samples tested positive for verotoxin by cell culture assay (47). Five O157:H7 and 38 non-O157 isolates representing 26 serogroups were recovered. Polymerase chain reaction analysis of fecal samples from 25 pens at 4 feedlots from southern Alberta identified samples from 20 of the pens that were positive for the genes which code for verotoxin, intimin, and the O26:H11 serogroup (48). Samples from 5 pens were positive for the genes coding for verotoxin, intimin, and the O111:H8 serogroup, while samples from only 2 pens were positive for the genes for the O157:H7 serogroup. In a second study of 84 pens at 21 feedlots in Alberta, non-O157 VTEC were recovered from samples from 12 of the pens. Polymerase chain reaction analysis of enrichment broths from 40 swabs from animals previously held in each of the pens showed that 7% of the broths were positive for verotoxin genes, *eae*, and *ehxA* although no VTEC were recovered from any of the 1650 samples (49).

Although PCR is the most commonly used method of screening for VTEC, enzyme-linked immunosorbent assay (ELISA) for verotoxin may be more sensitive. Screening of rectal feces from 500 Ontario beef cattle at slaughter, for verotoxin by ELISA or by PCR for the genes *vt1*, *vt2*, and *eae* found a rate of 10.2% positive by ELISA, but a rate of only 6.2% by PCR (50). Isolates belonging to 24 serogroups were recovered from 27 of the positive samples, but no O157:H7 were recovered.

Comparable results have been obtained in American studies. Of 334 swab samples taken from skinned beef carcass before evisceration (preevisceration) at 4 large packing plants in the USA, 25.4% were positive for O157:H7 VTEC alone, 21.0% were positive for non-O157 VTEC only, and 32.9% were positive for both non-O157 and O157:H7 VTEC (51). In the same study, 361 non-O157 VTEC isolates comprising 41 serogroups were recovered. A study conducted at 3 midwestern USA beef packing plants found that the prevalence of O157:H7 in fecal swab, hide, and preevisceration carcasses samples were 5.9%, 60.6%, and 26.7%, respectively. The prevalence of non-O157 VTEC was 34.3% in fecal swab samples, 92.0% in hide samples, and 96.6% in preevisceration carcasses samples (52). Cobbold et al (53) sampled 86 beef carcasses from Washington State and reported that 23.3% were positive for verotoxin genes by PCR analysis. Of the isolates were recovered from 6 samples, 5 were non-O157 and 1 was O157:H7 VTEC.

Non-O157 VTEC in Canadian and American beef

The prevalence of non-O157 VTEC in Canadian beef is difficult to estimate as very few studies on this topic have been reported. However, beef is undoubtedly contaminated with non-O157 VTEC as well as with other *E. coli*, by transfer of bacteria from the hide and equipment during carcass dressing and processing (54). Thus, the prevalence of non-O157 contamination can be assessed by reference to studies of O157:H7 and non-O157 VTEC prevalence in cattle before and after slaughter. Conclusions about the conditions of cattle and products in the Canadian beef industry can reasonably be drawn from studies conducted in the USA, because of the extensive integration and similarity of practices in the beef industries of the 2 countries.

The prevalence of non-O157 VTEC in Canadian beef appears to be at least equal to and is possibly greater than that of O157:H7 VTEC. A 1990 study found that of 225 samples of ground beef from Ontarian meat processing plants, 82 (36.4%) were positive for verotoxin production by vero cell assay. Verotoxigenic *E. coli* were isolated from 10.4% of the positive samples, and none of the isolates was of the O157 serotype (55). Testing of 400 ground beef samples from Calgary and Winnipeg in 1989–1990 recovered 8 O157:H7 isolates and 18 non-O157 isolates (56). A further 66 samples were verotoxin positive by cell culture assay, but no isolates were recovered.

The results of testing by the USDA-FSIS provide an accurate picture of the contamination of ground beef with *E. coli* O157:H7 in the USA. Testing of 26 521 samples obtained during the fiscal years 2000 through to 2003 found a prevalence rate of 0.71% (57). As in Canada, little information is available on the prevalence of non-O157 VTEC, though the limited evidence indicates that there is a higher prevalence of total VTEC than that reported for O157:H7 VTEC alone. Samadpour et al (58) reported that 16.8% of 296 ground beef samples collected in Washington State contained VTEC, although there was no attempt at discrimination between O157:H7 and non-O157 serogroups.

In a 2nd study of beef carcasses and finished product conducted in Washington State, 36% of 480 beef samples were positive for VTEC by PCR analysis of enrichment broth (53). Fifty VTEC isolates were recovered from 9.2% of samples; only one of which was of the O157:H7 serogroup. It was suggested that the significant difference between the number of PCR positive samples and the isolation rate was due to inhibition of non-O157 VTEC by the antimicrobials in the selective media used for isolation.

Interventions to control non-O157 VTEC in cattle and on beef

Effective methods for the control of carriage and shedding of VTEC by cattle would be desirable, to reduce both the numbers on hides available for transfer to beef during carcass dressing, and the numbers in the environment that may contaminate water or vegetables. Investigations of the means of controlling VTEC in cattle as yet have only been concerned with *E. coli* O157:H7. Thus, the effects of possible interventions on non-O157 VTEC must be deduced from the available information on control of O157:H7 VTEC.

Good farm management practices that limit the number of VTEC in animal feed and water might reduce the prevalence of the organisms in cattle (59); and good management of stock on farm, during transport and in lairages might reduce the spread of VTEC between

animals (60). It has been claimed that manipulation of cattle diets for limited periods before slaughter can reduce shedding of VTEC, but such claims have not been substantiated (61). Withdrawal of feed before slaughter, however, can lead to increased shedding of *E. coli* that presumably can include VTEC (62).

Various types of feed supplement have been shown to reduce shedding of O157:H7 VTEC under experimental conditions. The effective supplements include probiotic lactobacilli (63); non-pathogenic *E. coli* that can competitively exclude O157:H7 VTEC (64); cocktails of bacteriophage that infect and lyse O157:H7 VTEC (65); and sodium chlorate, which facultative anaerobes such as *E. coli* metabolize to bacteriocidal chlorite (66). Feed supplements of these sorts have effects on *E. coli* generally rather than on VTEC specifically. They might then be useful for controlling both non-O157 and O157:H7 VTEC. However, whether or not such treatments would be effective when used in commercial circumstances has yet to be demonstrated.

Vaccination of cattle with proteins secreted by the type III secretion system (TTSS) of the LEE (EspA, EspB and Tir) has been shown to reduce shedding of O157 VTEC (67). However, a subsequent study found that a vaccine based on TTSS secreted proteins from O157:H7 provided very limited cross protection against other VTEC serogroups (68). Thus, it would appear that vaccines based on TTSS secreted proteins would be effective against only a limited range of specific serogroups, unless universal chimeric proteins can be devised. Additionally, it can be assumed that vaccines targeting these proteins will have no impact on LEE negative VTEC.

Because VTEC as well as non-pathogenic *E. coli* are normal components of the gut flora of cattle, the effect of any treatment to control their numbers in cattle is likely to be limited. Moreover, consistent application of a treatment to all cattle cannot be expected. Thus, there is no reason to suppose that it would be possible to eliminate VTEC from the Canadian cattle herd. Facilities for transporting and holding cattle will then continue to be contaminated with VTEC, irrespective of any anti-VTEC treatments widely used with cattle in the future. Consequently, it is likely that the hides of most animals presenting for slaughter will continue to be contaminated with VTEC acquired from other animals or persistent sources of contamination in transport and holding facilities (69,70) as well as with any such organisms they may be shedding themselves.

Some of the VTEC present on cattle hides will inevitably be transferred to the meat during carcass skinning operations (54). Washing of carcass hides in automatic washing cabinets before skinning of carcasses has been reported to reduce the numbers and prevalence of O157:H7 VTEC on both hides and skinned carcasses (71,72); even though washing animals before slaughter has been found to have no, or deleterious, effects on the general microbiological conditions of carcasses (73). Whether or not washing hides is effective, washing skinned but uneviscerated or dressed carcasses can reduce the numbers of *E. coli*, presumably including VTEC, by about an order of magnitude, if the numbers are relatively high (74). However, if the numbers are relatively low, washing will have no effect. Spraying carcasses with antimicrobial solutions, cleaning small visibly contaminated sections of carcasses with hot water delivered from spray nozzles within an operating vacuum head, and trimming of visibly contaminated tissues all have negligible effects on the microbiological conditions of carcasses (75). However, pasteurizing dressed carcass sides with hot water at temperature $> 80°C$ or steam at temperatures about 100°C can reduce the numbers of *E. coli* by more than 2 orders of magnitude, to give carcasses on which *E. coli* are present at numbers of $< 1/1000$ cm^2 (76,77). The numbers of VTEC on such carcass sides must be disappearingly few.

Meat from carcasses that are largely free of *E. coli* can be recontaminated with organisms during carcass breaking processes (78). The contaminants are derived from detritus that persists after routine cleaning, on parts of equipment that do not contact meat. Such areas include the undersides and drive mechanism of conveyor belts. The persistent detritus will be at temperatures that allow proliferation of *E. coli* during the daily cleaning of carcass breaking facilities, and may be warmed to such temperatures by frictional heating during operation of the equipment in the cooled carcass breaking facilities. As the *E. coli* in detritus can include VTEC as well as non-pathogenic strains, most of the VTEC on beef from some plants may originate from such sources (79).

The presence of both O157:H7 and non-O157 VTEC on beef could be dramatically reduced if carcass-dressing processes were arranged to minimize microbiological contamination of the meat, carcasses were subjected to effective pasteurizing treatments, and the microbiological condition of the pasteurized meat was maintained during carcass breaking. Although this would seem to be possible in commercial practice (54), few plants worldwide are preparing beef compliant to this high hygienic standard.

In conculsion, the available Canadian and US studies show that VTEC of serogroups other than O157:H7 are a significant source of human illnesses in Canada, and possibly cause up to 50% of all VTEC illnesses. Since there is no necessary relationship between serogroup and pathogenicity in *E. coli*, serogrouping can not be relied upon as a method for identifying VTEC. However certain serogroups have a higher association than others with VTEC illness in humans. In the USA, the serogroups O26, O111, O103, O121, O45, and O145 account for 70% of non-O157 VTEC illness and a similar situation is likely to exist in Canada. The only verified determinant of pathogenicity in VTEC is the secretion of verotoxin. Therefore, to determine whether *E. coli* isolates are VTEC, verotoxin production or genes for its production must be confirmed.

A wide range of VTEC serogroups have been isolated from Canadian and US cattle and the prevalence of non-O157 VTEC appears to be as high as that of O157:H7 VTEC. Because of the ubiquitous presence of VTEC in cattle and their environment, preslaughter management and/or treatment of cattle might reduce, but will not eliminate VTEC. Consequently, the hides of most if not all cattle presenting for slaughter are likely to be contaminated to some extent with either or both O157:H7 and non-O157 VTEC acquired from transport and lairage environments. However VTEC contamination of beef can be effectively addressed by good management of carcass dressing processes and pasteurizing of dressed carcass sides, as these practices can give carcasses that are essentially free of VTEC. Beef from carcasses that are free of VTEC may be recontaminated, but recontamination can be reduced or prevented by appropriate management of the carcass-breaking process.

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