

# Enhanced surveillance of non-O157 verotoxin-producing *Escherichia coli* in human stool samples from Manitoba

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LH Thompson, S Giercke, C Beaudoin, D Woodward, JL Wylie. Enhanced surveillance of non-O157 verotoxin-producing *Escherichia coli* in human stool samples from Manitoba. *Can J Infect Dis Med Microbiol* 2005;16(6):329-334.

**BACKGROUND:** Relatively few enhanced surveillance studies have been undertaken to investigate the extent to which verotoxin-producing non-O157 serotypes of *Escherichia coli* occur in stool samples received for the detection of verotoxin-producing organisms.

**OBJECTIVES:** To describe the prevalence, molecular and epidemiological characteristics, and geographical patterns associated with non-O157 verotoxin-producing *E coli* (VTEC) in Manitoba.

**RESULTS:** Thirty-two VTEC isolates consisting of 10 serogroups and 13 different serotypes were isolated over a 22-month period. Twenty-three isolates (71.8%) possessed verotoxin-encoding gene *stx1* only, five isolates (15.6%) possessed *stx2* only, two isolates (6.3%) possessed both *stx1* and *stx2*, and two isolates (6.3%) possessed *stx2c*. Only three instances of indistinguishable pulsed-field gel electrophoresis patterns were identified. The age of the individuals from whom non-O157 VTEC were isolated ranged from eight months to 87 years. Mean and median ages were 30 and 22 years of age, respectively. Some areas of the province appeared to experience a higher than expected number of non-O157 *E coli* in comparison with the number of stools that were received from these areas.

**CONCLUSIONS:** The present study demonstrated a large number of infections associated with non-O157 VTEC in Manitoba. Most non-O157 cases appear to result from sporadic infections, and these occur typically in rural areas. Continued enhanced surveillance is necessary to understand the temporal patterns of non-O157 VTEC and the underlying epidemiological factors driving these patterns.

**Key Words:** *E coli*; Epidemiology; Non-O157; Surveillance; Verotoxin

There is increasing recognition that verotoxin (VT)-producing non-O157 serotypes of *Escherichia coli* have been underappreciated as a human pathogen. Historically, the ease of identifying O157:H7 *E coli* by its inability to ferment sorbitol, its association with several large, well-publicized outbreaks, and its clear association with severe clinical syndromes has resulted in diagnostic efforts being focused on this serotype. However, enhanced surveillance in Nebraska and Montana (1,2) and an earlier analysis by Cadham Provincial Laboratory (CPL) in Winnipeg, Manitoba, have demonstrated that approximately

## Surveillance accrue des sérotypes d'*Escherichia coli* non O157, producteurs de vérotoxine dans des échantillons de selles, prélevés chez des humains, au Manitoba

**CONTEXTE :** Un nombre relativement restreint d'études de surveillance accrue a été mené pour déterminer la fréquence des sérotypes d'*Escherichia coli* non O157, producteurs de vérotoxine; aussi a-t-on reçu des échantillons de selle dans le but de détecter la présence de micro-organismes producteurs de vérotoxine.

**BUT :** L'étude visait à décrire la prévalence, les caractéristiques moléculaires et épidémiologiques ainsi que la répartition géographique des souches d'*E. coli* non O157, productrices de vérotoxine (ECPV), au Manitoba.

**RÉSULTATS :** Trente-deux isolats d'ECPV, constitués de 10 sérogroupes et de 13 sérotypes ont été identifiés sur une période de 22 mois. Vingt-trois isolats (71,8 %) contenaient le gène codant de la vérotoxine *stx 1* seulement; cinq (15,6 %), le gène *stx 2* seulement; deux (6,3 %), les gènes *stx 1* et *stx 2*; et deux autres (6,3 %), le gène *stx 2c*. Il n'y a eu que trois cas de diagramme électrophorétique sur gel en champ pulsé, non reconnaissable. L'âge des personnes chez qui des isolats d'ECPV non O157 ont été identifiés variait de 8 mois à 87 ans. L'âge moyen et l'âge médian se sont établis à 30 ans et à 22 ans respectivement. Certaines régions de la province ont semblé connaître un nombre de cas d'*E. coli* non O157 plus élevé que prévu par rapport au nombre de prélèvements que celles-ci avaient fait parvenir.

**CONCLUSIONS :** La présente étude révèle un nombre élevé d'infections associées à des souches d'ECPV non O157, au Manitoba. La plupart des cas semblent liés à des infections sporadiques et ils s'observent surtout en région rurale. Une surveillance accrue et continue s'impose pour nous permettre de mieux comprendre l'évolution des infections associées aux souches d'ECPV non O157 dans le temps ainsi que les facteurs épidémiologiques sous-jacents.

50% of VT-producing *E coli* (VTEC) in human diarrheal stool samples in these areas are non-O157 serogroups (3). This pattern fits with data from Europe and Australia, where non-O157 VTEC predominate. In these geographical locales, non-O157 VTEC, like O157 VTEC in North America, have been associated with large outbreaks and severe sequelae, such as hemorrhagic colitis and hemolytic uremic syndrome (HUS).

In general, VTEC infections share several common epidemiological patterns. They typically originate from food, water and zoonotic sources or by human-to-human transmission.

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Received for publication April 14, 2005. Accepted September 19, 2005

Predominant animal reservoirs include cattle and sheep, and common food-borne transmission involves the consumption of undercooked meat and water contaminated by ruminant feces (4). In humans, VTEC may be asymptomatic or associated with gastroenteritis with or without bloody diarrhea. As noted above, infections may progress to more severe syndromes including hemorrhagic colitis, HUS and thrombotic thrombocytopenic purpura, with children and the elderly showing the greatest susceptibility (5).

At the molecular level, virulence factors vary within the different VTEC serogroups. This characteristic is especially true for VT, the toxin produced by all VTEC. VT is encoded by the genes *stx1* and *stx2*. Although the products of both genes have a similar enzymatic action and structure, the cytotoxicity of the *stx2* product relative to the *stx1* product has been estimated as 1000-fold greater, with a stronger association existing between *stx2*-containing VTEC and a severe clinical outcome. Large outbreaks of non-O157 containing *stx2* (eg, the O26 serogroup in some European countries) have been recorded. Several variants of *stx2* are known, including *stx2c*, *stx2d*, *stx2e* and *stx2f* (6). Many VTEC also harbour other virulence factors, such as the *eaeA* gene, which produces intimin, and the *hlyA* gene, which encodes enterohemolysin (6).

In 2002, we began to systematically isolate all VTEC from VT-positive human stool specimens received at CPL because of the relative paucity of data on non-O157 serogroups in North America. The focus of the present paper is on non-O157 VTEC. These data provide an opportunity to review temporal trends in our jurisdiction and conduct a more detailed molecular and epidemiological analysis of the non-O157 serogroups currently present in the province.

## MATERIALS AND METHODS

### Bacterial strains and culture media

The 32 human isolates of VTEC included in the present study were isolated from human stool specimens received at CPL. Specimen collection guidelines for CPL requires that stool specimens be shipped to the laboratory without transport media. Stools are examined for *Salmonella*, *Shigella*, *Yersinia*, *Aeromonas* and *Campylobacter*. Outbreak-associated stools are also examined for *Staphylococcus aureus*, *Bacillus cereus*, *Clostridium perfringens* and *Listeria monocytogenes*. All liquid or bloody stools are examined using a cytotoxin assay for the presence of VT or verotoxigenic organisms. During outbreak investigations, all stools are examined for VT activity, regardless of consistency.

The cytotoxin assay in routine use at CPL detects either free fecal VT (FVT) or VT produced after polymyxin extraction (PECS) (7). For FVT, stools were diluted (a ratio of one volume of stool to four volumes of phosphate buffered saline; or a 1:3 dilution for very watery stools). For both FVT and PECS, HeLa cells were examined for the characteristic rounding morphology exhibited in response to the presence of VT (8). The presence of VT was confirmed by neutralization of VT activity with rabbit polyclonal antibodies raised against VT1 and VT2.

Systematic attempts to recover the VT-producing organism from all VT-positive stools began in 2002. For purposes of the present study, organisms isolated from June 2002 to April 2004 were included. An inoculum from VT-positive PECS was plated to MacConkey agar with sorbitol (SMAC) and examined for the presence of nonsorbitol fermenting (NSF) colonies. Any NSF colonies were purified and assayed for VT production. If VT

production was confirmed, then isolate identification was confirmed by the Vitek II system (bioMérieux, Canada).

If no NSF colonies were present on SMAC, but the plate appeared to contain a relatively 'pure' (by colony appearance) culture of sorbitol fermenters, then one colony was selected and examined for VT production by the cytotoxin assay. If VT-producing *E coli* was not identified at this stage, or if the original SMAC plate contained numerous colony morphologies, then additional media, including Rainbow Agar (Biolog Inc, USA) and CHROMagar O157 (Becton Dickinson, Canada) were used to aid in recovery and identification. In practice, approximately 50% of the VT-positive stools will result in SMAC plates that appear to contain a nearly pure culture of either NSF or SF colonies. For the remaining stools, a VT-producing isolate can usually be found using CHROMagar (O157:H7 appear as mauve cells, while non-O157 VTEC typically present as either pink or blue colonies). The need to resort to the use of Rainbow Agar is rare. Overall, this algorithm is able to identify a VT-producing organism from approximately 90% of VT-positive stools. As is typical for most diagnostic laboratories, given the inherent time constraints, all isolation attempts are stopped once a VT-producing isolate is identified at any of the above steps (eg, attempts to demonstrate mixed infections are not pursued). Serotyping of purified isolates was performed as part of the Enteric Diseases Program at the National Microbiology Laboratory (Public Health Agency of Canada, Winnipeg, Manitoba).

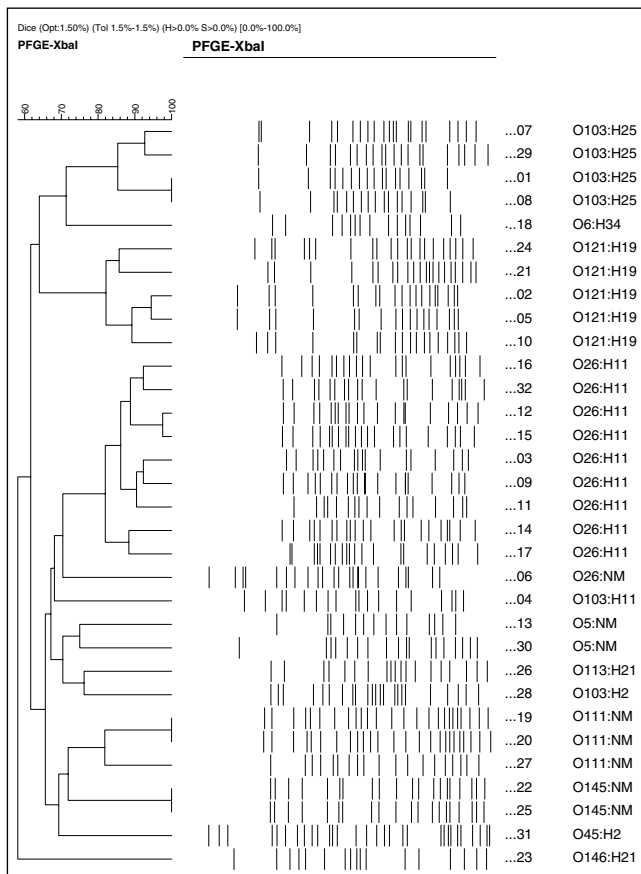
### Polymerase chain reaction amplification methods

Total genomic DNA was isolated from bacterial colonies grown overnight on split blood/trypticase soy agar. Bacterial suspensions were lysed with lysozyme (100 ng/μL final concentration; Sigma, Canada). Following a 30 min incubation at 37°C, proteinase K (Sigma, Canada) was added to a final concentration of 80 ng/μL. Cells were digested for 30 min at 50°C, followed by 10 min at 100°C.

Isolates were screened by polymerase chain reaction (PCR) for VT-encoding genes *stx1*, *stx2*, *stx2c*, *stx2d*, *stx2e* and *stx2f*, and the intimin (*eaeA*) and plasmid-encoded enterohemolysin (*hlyA*) genes. With minor modifications, the previously described procedure of Wang et al (9) was used. The three sets of primer mixtures were used as described, with the exception that the primers for *rfbE*<sub>O157</sub> and *fliC*<sub>H7</sub> encoding the *E coli* somatic antigen O157 and structural flagellar antigen H7 were not used. In all other respects, the three separate primer mixes were formulated as described, including the use of the E16s primers (targeting the 16 s ribosomal RNA gene) as an internal amplification control. The 100 μL multiplex PCR reaction mixes contained 10 μL of 10× Dynazyme PCR buffer (Bio-Rad Laboratories, Canada), 0.2 mM deoxynucleotides (Amersham Biosciences, Canada) and 2.5 U of Dynazyme DNA polymerase (Bio-Rad Laboratories). Primers were synthesized by the University Core DNA Services, University of Calgary (Calgary, Alberta). DNA amplification was performed in a Perkin Elmer GeneAmp PCR System 9600 (PerkinElmer, USA) using thermocycling conditions as described elsewhere (9), with the exception of an initial denaturation at 95°C for 5 min. PCR products were visualized on 1.0% agarose gels.

### Pulsed-field gel electrophoresis

Pulsed-field gel electrophoresis (PFGE) of *E coli* followed the standardized protocol as adopted by the National Microbiology Laboratory and originally developed for PulseNet by the Centers for Disease Control and Prevention in Atlanta, Georgia, USA (10). Electrophoresis of the *XbaI*-digested DNA used a



**Figure 1** Pulsed-field gel electrophoresis (PFGE) fingerprint patterns of verotoxin-producing *Escherichia coli* isolates, arranged according to BioNumerics (software version 3.5, Applied Maths, USA) cluster analysis with pairwise similarity matching, using the Dice band-based and unweighted pair group method with arithmetic averages as the dendrogram type. Each pattern is identified by an isolate number and its serotype

CHEF-DR II (Bio-Rad Laboratories) with 1.0% SeaKem gold agarose (Mandel Scientific Company, Canada). Gel images were scanned with a Kodak EDAS system (Eastman Kodak Company, USA). Results were analyzed using BioNumerics software (version 3.5, Applied Maths, USA). The dendrogram shown in Figure 1 was constructed using BioNumerics, with the Dice coefficient and the unweighted pair group method with arithmetic averages (UPGMA) set with optimization and position tolerances of 1.0%.

### Geographical analysis

SaTScan software version 4.0.3 (11) was employed for a spatial analysis of non-O157 VTEC in Manitoba. The authors previously used this approach to analyze the distribution of *Chlamydia trachomatis* genotypes in Manitoba (12). The province of Manitoba was divided into 11 geographical units based on the boundaries of the 11 regional health authorities (RHAs): Assiniboine, Central, Interlake, North Eastman, South Eastman, Parkland, Norman, Burntwood, Churchill, Brandon and Winnipeg. The latitude and longitude chosen for each geographical unit represents the approximate geographical centre of each area. The residence location chosen for each patient record was aggregated to the RHA level.

The underlying statistical methods for SaTScan have been published by the software developers (13,14). Two models are

**TABLE 1**

**Summary of the number of verotoxin-producing non-O157 *Escherichia coli* isolates identified and their molecular characteristics**

Serotype*	Number of isolates	Genes detected							
		<i>hlyA</i>	<i>eaeA</i>	<i>Stx1</i>	<i>Stx2</i>	<i>Stx2c</i>	<i>Stx2d</i>	<i>Stx2e</i>	<i>Stx2f</i>
O26:H11	9	+	+	+	-	-	-	-	-
O121:H19	5	+	+	-	+	-	-	-	-
O103:H25	4	+	+	+	-	-	-	-	-
O145:HNM	2	+	+	+	-	-	-	-	-
O5:HNM	2	+	+	+	-	-	-	-	-
O111:NM	2	+	+	+	+	-	-	-	-
O111:NM	1	+	+	+	-	-	-	-	-
O146:H21	1	+	+	+	-	-	-	-	-
O103:H11	1	+	+	+	-	-	-	-	-
O45:H2	1	+	+	+	-	-	-	-	-
O103:H2	1	+	+	+	-	-	-	-	-
O26:HNM	1	+	+	+	-	-	-	-	-
O6:H34	1	-	-	-	-	+	-	-	-
O113:H21	1	+	-	-	-	+	-	-	-

\*Serotypes are ranked in order from the most common to the least common

available for spatial analysis in SaTScan – Bernoulli and Poisson; both are approximations for each other when conducting spatial analysis (as opposed to spatial-temporal). The advantage of using Poisson is that covariates can be adjusted for (11). SaTScan software settings used in the present analysis were as follows: type of analysis – spatial; probability model – Poisson; coordinates – latitude/longitude; maximum spatial cluster size – 50%. This latter setting governs the maximum size of identified clusters. A setting of 50% indicates that clusters could, at most, contain 50% of the provincial population.

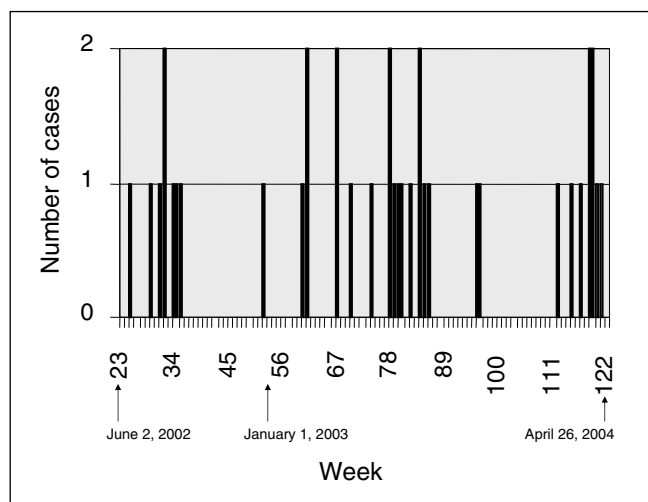
## RESULTS

### Serotyping

The 32 non-O157 VTEC isolates consisted of 10 serogroups with 13 different serotypes (Table 1). The four most common serotypes were O26:H11 (nine isolates), O121:H19 (five isolates), O103:H25 (four isolates) and O111:NM (three isolates). Two serotypes were represented by two isolates each (O5:HNM and O145:HNM). Single isolates were seen for the remaining serotypes. For approximately the latter half of the investigational period (May 2003 to April 2004), data on the ratio of non-O157 versus O157 serotypes were available. In Manitoba, during this time frame, 19 of 30 (63%) VTEC were non-O157. Although several more years of surveillance would be necessary to increase the accuracy of this estimate, the current data highlight the large number of VT-related cases associated with non-O157 serotypes.

### Virulence gene typing

The *hlyA* gene was present in 97% (31 of 32) of the non-O157 VTEC. The serotype lacking this gene was O6:H34. This isolate and the O113:H21 isolate were unique in containing *stx2c* and were also the only isolates lacking *eaeA*. The five O121:H19 isolates each possessed *stx2*, while two of the three O111:NM isolates possessed both *stx1* and *stx2*. The remaining serotypes each possessed *stx1* only. The genes *stx2d*, *stx2e* and *stx2f* were not detected in any of the isolates. In total, 23 (71.8%) isolates possessed *stx1* only, five (15.6%) possessed *stx2* only, two (6.3%) possessed both *stx1* and *stx2*, and two (6.3%) isolates possessed *stx2c*.



**Figure 2)** Time frame for identification of non-O157 verotoxin-producing *Escherichia coli* cases (based on date that specimens were received). The investigational period covered 99 weeks (June 2, 2002, to April 26, 2004)

### PFGE

A dendrogram showing the results of the PFGE analysis is shown in Figure 1. For serotypes with multiple isolates, all of the isolates within a given serotype clustered together on the dendrogram. Only three instances of indistinguishable PFGE patterns were identified. These included two isolates each within the serotypes O111:HNH, O103:H25 and O145:HNH.

### Epidemiology

The age of the individuals from whom non-O157 VTEC were isolated ranged from eight months to 87 years. Mean and median ages were 30 and 22 years of age, respectively. Eighteen of the 32 (56%) individuals were male. HUS is a reportable disease in Manitoba and the case names associated with the non-O157 isolates analyzed in the present paper were checked against HUS-reported cases. None of the non-O157 cases were associated with HUS.

None of the three pairs of isolates with indistinguishable PFGE patterns noted above appeared to involve sibling pairs (based on different surnames and residence in different geographical areas of the province). These data suggest that some non-O157 case clusters may have originated from a common source within the time frame of the study.

The time frame for the isolation of the non-O157 cases analyzed is shown in Figure 2. Although additional data from subsequent years would be necessary to identify clear trends, late fall/early winter was associated with the fewest cases of non-O157 VT-producing isolates in the data set.

### Geography

The geographical distribution of the non-O157 VTEC cases was examined to determine whether higher-than-normal rates of this pathogen occurred in any part of the province. Of the 32 cases, 19 were identified as forming a cluster. The SaTScan output indicated that 9.25 cases would be expected in the area encompassed by this cluster if cases were distributed randomly across the province ( $P < 0.006$ ). This cluster encompassed the cases reported in five adjoining RHAs in the southeast and central parts of the province: Parkland, Interlake, Assiniboine,

Brandon and Central. Within these RHAs, most cases were from rural areas or small towns. Only one of the 19 cases resided in a relatively large population centre (population 40,000). The largest city in Manitoba (Winnipeg, population 650,000) did not form part of the cluster. No areas of the province were identified as having lower-than-expected rates of non-O157 VTEC.

To determine whether this clustering pattern simply reflected the catchment area for receipt of stools at CPL (eg, a higher-than-expected number of cases may simply reflect a large number of stool specimens being sent to CPL from those areas), SaTScan analysis was repeated using the total number of stools tested at CPL for the presence of verotoxin, stratified by RHA. The time frame for this dataset matched the time frame covered by the non-O157 VTEC isolates used in the present study (June 2002 to April 2004). A cluster was identified that encompassed six RHAs in northern and central Manitoba: Burntwood, Norman, Parkland, Interlake, North Eastman and Central. The case clusters and total specimens clusters overlapped in the central part of the province (Parkland, Interlake and Central). The higher-than-expected number of cases found in these three RHAs may therefore simply reflect the high number of stool specimens received from these areas. The inclusion of two RHAs in the southwestern part of Manitoba (Brandon and Assiniboine) in the case cluster but not the total specimens cluster suggests these areas may experience a higher-than-expected number of non-O157 *E. coli* in comparison with the number of stools received from these RHAs. Conversely, the absence of Burntwood, Norman and North Eastman from the case cluster, coupled with their inclusion in the total specimens cluster, suggests these areas may experience a lower number of non-O157 *E. coli* in relation to the number of stools forwarded from these areas. Geographical analyses of this type are useful to help identify areas where the incidence of a disease seems unusually high. Data of this type can drive further investigational efforts to determine whether the underlying epidemiology in these areas differs from areas where the incidence is lower.

### DISCUSSION

This paper presents the results of an enhanced surveillance effort conducted on non-O157 VTEC in Manitoba. Two earlier Canadian studies provide comparative reference points for our results. Canadian VTEC data from 1983 to 2000 (15) provide an opportunity to compare our local regional results to overall national results. A surveillance study done in Manitoba in 1992 by CPL (3) provides an opportunity to examine non-O157 temporal patterns using data acquired with similar surveillance techniques.

Cumulative Canadian data from 1983 to 2000 show that the most frequently occurring serotype identified in Manitoba (O26:H11) was the most common non-O157 VTEC serotype from human sources overall in Canada. All of the serotypes we identified have been previously associated with human cases in Canada, with one exception (O6:H34). The O6:H34 isolate has been identified in Canadian cattle. Because many laboratories do not routinely check for all types of VTEC, there is a clear difference between the ratio of non-O157 and O157 VTEC historically sent to the National Microbiology Laboratory (4.6% [15]) versus those found by active surveillance (48% in Manitoba in 1992 [3] and 63% in the present study). Our relative prevalence data are similar to those seen in



two American states and are likely a more accurate reflection of the non-O157 prevalence in North America.

The six serotypes of non-O157 VTEC identified in Manitoba from 1983 to 2000 were identified again in Manitoba during our enhanced surveillance with approximately the same relative prevalence (eg, the three most common serotypes in Manitoba from 1983 to 2000 remain the most common Manitoba serotypes in the enhanced surveillance of 2002 to 2004). Our local prevalence data differ from overall Canadian results, where serotypes O128:H12, O126:H8 and O1:H7 are second, third and fourth most common after O26:H11. This disparity results from regional differences within Canada with respect to the most common serotype present. If these regional differences persist over time (as the comparison between historical Manitoba data and our current surveillance data suggests), then non-O157 VTEC may generally show localized endemic transmission within relatively small geographical areas. Confirmation of this possibility would require more detailed temporal surveillance of non-O157 from a larger number of distinct geographical locales.

In contrast to the relative stability in serotype prevalence implied by comparing our results with historical cumulative Manitoba data, a comparison of our results with detailed Manitoba results from 1992 suggests temporal fluctuation in the prevalence of some serotypes. In 1992, over a seven-month period, VTEC were isolated from VT-positive stools at CPL (3) using techniques similar to those we used for the 2002 to 2004 surveillance period. Although several serotypes were seen in both time periods, two of the most frequently isolated serotypes in the present study (O26:H11 and O103:H25) were not identified in 1992. This pattern may reflect the limitations imposed by the shorter sampling period in 1992 (in 1992, a total of only 23 VTEC [12 O157 and 11 non-O157] were isolated), the emergence of these serotypes in Manitoba since 1992, or continual fluctuations in prevalence over time. The latter possibility may be correct, given that our current study period covered two complete summer seasons, yet the majority of the O26:H11 isolates were seen in 2003 (seven of nine). Based on PFGE data, the increase seen in 2003 did not appear to be due to a single strain outbreak in that year. Additional research will be necessary to fully understand the temporal prevalence patterns of non-O157 VTEC and their underlying epidemiology.

A Nebraska study and several studies (2,18,19) in Europe found that most non-O157 VTEC were sporadic infections. Our PFGE data also suggest that this is the case in Manitoba.

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The majority of serotypes with multiple isolates showed unique PFGE fingerprint patterns, suggesting that outbreaks of these organisms are not common. However, three pairs of isolates were identified with indistinguishable PFGE patterns. In each instance, the pairs of individuals were not obviously related to each other. In the absence of a thorough epidemiological investigation, the possibility of direct human-to-human transmission between these individuals cannot be ruled out; however, these data suggest that multiple infections of some non-O157 serotypes do originate from common sources.

A substantial number of the non-O157 isolates we identified were serotype O26:H11, with the majority of these being isolated within a five-month period. However, as noted above, our PFGE data did not support the occurrence of a common source outbreak of this serotype, unlike the outbreaks that have been linked to this serotype in some parts of Europe and Japan (16-18). One notable cluster in Germany was associated with an O26:H11 strain carrying *stx2* (unlike the *stx1* typically found in this serotype). O145 serotypes in Europe have also been reported as containing *stx2* (unlike the *stx1*-carrying O145 organisms seen in Manitoba). *stx2* is reported as having higher activity, and these different genetic profiles may play a role in the significance and clinical severity of the clinical presentations seen in different areas. In common with most areas, the majority of non-O157 serotypes in Manitoba contain *stx1* only. Studies in Germany and Austria, Finland, Montana, Nebraska, Spain and Calcutta (1,2,4,5,20,21) also revealed higher proportions of isolates with *stx1* rather than *stx2*.

## CONCLUSION

Our systematic efforts to isolate all VTEC from human stool samples continue to demonstrate the large number of infections associated with non-O157 VTEC and the importance of screening stools for the presence of VT, rather than screening for the presence of O157:H7 serotypes only. A comparison of historical data for Manitoba and Canada suggests relative stability of the serotypes within a region over long time frames, but also that the prevalence of common serotypes may fluctuate widely from year to year. Most non-O157 cases appear to result from sporadic infections and occur typically in rural areas. This latter observation suggests that an association with animals, rather than the consumption of animal products, may play a significant role in the epidemiology of these infections. Continued enhanced surveillance is necessary to understand the temporal patterns of non-O157 VTEC and the underlying epidemiological factors driving these patterns.

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