Prevalence and characterization of verotoxin-producing *Escherichia coli* (VTEC) in cattle from an Ontario abattoir

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Abstract

This study determined the prevalence of verotoxin (VT)-producing *Escherichia coli* (VTEC) in Ontario beef cattle at slaughter and characterized the isolates by serotype, virulence factors, virulence markers, and antimicrobial resistance. Cultures of rectal feces from 500 animals were screened for VT by an enzyme-linked immunosorbent assay (ELISA) and by polymerase chain reaction (PCR) for genes *vt1*, *vt2*, and *eae*. The VT-ELISA-positive samples were tested by a VT-immunoblot to isolate VTEC colonies. The prevalence rates of VTEC by VT-ELISA and PCR were 10.2% [95% confidence interval (CI), 7.8% to 13.2%] and 6.2% (95% CI, 4.4% to 8.7%), respectively. Colonies of VTEC were isolated from 27 (53%) of the 51 VT-ELISA-positive samples and belonged to 24 serotypes, which did not include O157:H7. Twelve of the serotypes have been implicated in disease in humans. Virulence profiling of the isolates by PCR revealed that 2 (8%) were *eae*-positive, 5 (21%) had *vt1* only, and 19 (79%) had *vt2*, of which 3 had *vt2* only, 7 had *vt1* + *vt2*, 4 had *vt2* + *vt2c*, 2 had *vt2* + *vt2c* + *vt2d*, 2 had *vt1* + *vt2* + *vt2c*, and 1 had *vt1* + *vt2* + *vt2c* + *vt2d*. The distribution of selected plasmid-encoded putative virulence genes was as follows: *ehxA*, 63%; *espP*, 46%; *saa*, 67%; and *subA*, 54%. Nine of the 24 isolates were resistant to 1 or more antimicrobials. Major conclusions are that the VTEC prevalence of 10.2% was among the lower rates reported for beef cattle, a high proportion of the isolates had *vt2* genes, the *subA* gene was reported for the 1st time in Canadian VTEC, and the absence of O157 VTEC likely reflects the use of a technique that detected all VTEC.

Résumé

Cette étude effectuée en Ontario visait à déterminer la prévalence des E. coli *producteurs de vérotoxine (VTEC) chez les bovins d'embouche au moment de l'abattage et de caractériser les isolats par sérotype, facteurs de virulence, marqueurs de virulence et résistance antimicrobienne. Les cultures d'échantillons de fèces rectales provenant de 500 animaux ont été vérifiées pour la présence de vérotoxine (VT) par ELISA et par réaction d'amplification en chaîne par la polymérase (PCR) pour les gènes des vérotoxines (*vt1 *et* vt2*) et l'intimine (*eae*). Les échantillons positifs par ELISA pour VT ont été testés par immunobuvardage afin d'isoler les colonies de VTEC. Les taux de prévalence de VTEC par ELISA-VT et PCR étaient respectivement de 10,2 % (IC 95 % 7,8 % à 13,2 %) et 6,2 % (IC 95 % 4,4 % à 8,7 %). Des VTEC ont été isolés de 27 (53 %) des 51 échantillons ELISA-VT positifs et appartenaient à 24 sérotypes, qui n'incluaient pas O157:H7. Douze des sérotypes ont été impliqués dans des pathologies humaines. Le profilage de virulence des isolats par PCR a révélé que 2 (8 %) étaient* eae*-positifs; cinq (21 %) portaient* vt1 *seulement, et 19 (79 %) portaient* vt2*, dont trois avaient* vt2 *seulement, sept avaient* vt1 + vt2*, quatre avaient* vt2 + vt2c*, deux avaient* vt2 + vt2c + vt2d*, deux avaient* vt1 + vt2 + vt2c*, et un avait* vt1 + vt2 + vt2c + vt2d*. La distribution de gènes de virulence putatifs sélectionnés et codés par des plasmides était :* ehxA*, 63 %;* espP*, 46 %;* saa*, 67 %; et* subA*, 54 %. Neuf des 24 isolats étaient résistants à un antimicrobien ou plus. Les principales conclusions sont à l'effet que la prévalence des VTEC de 10,2 % était parmi les taux faibles rapportés pour les bovins d'embouche, une forte proportion des isolats portaient le gène* vt2*, le gène* subA *est rapporté pour la première fois chez des isolats VTEC canadiens, et l'absence de VTEC O157 reflète probablement le fait qu'une technique détectant tous les VTEC était utilisée.*

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Introduction

Verotoxin (VT)-producing *Escherichia coli* (VTEC) are foodborne pathogens that cause diarrhea, hemorrhagic colitis, and hemolytic-uremic syndrome (HUS) in humans (1). Although VTEC-associated illness in humans has been attributed mostly to *E. coli* O157:H7, non-O157 VTEC can also cause disease and may be highly virulent (2–4). It has been estimated that 73 000 infections in humans are caused by *E. coli* O157:H7 annually in the United States, and a further 37 000 cases are caused by non-O157 VTEC (5). Cattle are considered to be the principal reservoir of VTEC that infect humans (1,3,6). Most disease outbreaks have been attributed to consumption of water or food contaminated by cattle feces (such as undercooked ground beef, unpasteurized dairy products, and vegetables) and to contact with

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cattle (1). More than 400 O:H VTEC serotypes have been isolated from cattle and humans (7). The fact that VTEC serotypes differ in both their frequency of association with disease in humans and the severity of disease suggests differences in virulence traits (3,8,9).

The capacity to produce 1 or more immunologically distinct VTs, VT1 and VT2, defines VTEC; VT2 and VT2c have been associated with a higher risk of HUS in humans (8,10). Some serotypes are able to induce a characteristic attaching and effacing (A/E) lesion on intestinal epithelia (1). Formation of the A/E lesion is determined by a pathogenicity island known as the locus of enterocyte effacement (LEE) and is dependent on the injection of bacterial effectors into host cells via a type-III secretion system (1). The LEE-encoded adhesin intimin (Eae) and the translocated intimin receptor (Tir) are required for colonization of calves and adult cattle by O157:H7 VTEC, although non-LEE-encoded factors contribute as well (11). Intimin has been strongly associated with a higher risk of hemorrhagic colitis in humans (2). Genes on a 90-kb plasmid (pO157) in VTEC O157:H7 encode a number of putative virulence factors, including a hemolysin *(ehxA)*, a catalase peroxidase *(katP)*, a serine protease *(espP)*, and a type-II secretion system *(etp)* (12–14). Genes for other plasmid-encoded putative VTEC virulence factors include *saa*, which encodes an auto-agglutinating adhesin, and *subA* and *subB*, which encode a subtilase cytotoxin recently reported for VTEC O113:H21 and a number of other VTEC serotypes in Australia (15,16).

In this study, bovine fecal samples were collected from a southern Ontario abattoir to determine the prevalence of VTEC in Ontario beef cattle and to characterize the VTEC isolates with respect to serotype, major virulence factors, other virulence markers, and antibiotic resistance profile.

Materials and methods

Sample collection

Samples were collected from a large federally inspected abattoir in southern Ontario that slaughters on average 1000 young adult fat beef cattle per day. The cattle processed at this plant originate from the United States and several provinces of Canada, but samples were collected from Ontario animals only. Rectal fecal samples were collected weekly over a 20-wk period from April to October 2004. To ensure sampling of several lots and to minimize within-lot clustering, every 20th animal was sampled, for a total of 25 animals at each visit. Feces (approximately 25 g) were obtained at the evisceration table through a cut in the rectum and transferred to a 50-mL sterile polypropylene container (Fisher Scientific, Nepean, Ontario). The samples were placed on ice, transported to the laboratory, and frozen at −20°C until further processed.

Sample testing

The prevalence of VTEC was estimated by testing enrichment cultures of fecal samples for the presence of VTs by an enzyme-linked immunosorbent assay (ELISA) that detects all VTs (17) and by a polymerase chain reaction (PCR) for *vt* genes (18). Each fecal sample was thawed at room temperature, and 5 g of feces was added to 45 mL of modified EC broth (Difco, Detroit, Michigan, USA) with novobiocin (19) and incubated overnight at 37°C. A 1-mL aliquot of the broth culture was centrifuged at 10 000 × *g* for 5 min, and the pelleted cells were tested for *vt1*, *vt2*, and *eae* by PCR (18). The supernatant was tested by the VT-ELISA (17), and positive broths were processed by the VT colony immunoblot technique to isolate VTEC colonies (17). Isolated colonies were serotyped and then characterized by PCR for virulence factors and markers. The 95% confidence intervals (CIs) for the point estimates of prevalence by VT-ELISA and PCR were determined by the Wald method (20). The kappa value (coefficient of agreement) was calculated to establish the agreement beyond chance between the PCR and VT-ELISA results.

Characterization of VTEC isolates

The VTEC isolates were serotyped by standard procedures at the *E. coli* Reference Laboratory of the Laboratory for Foodborne Zoonoses, Public Health Agency of Canada, Guelph, Ontario, and were characterized by PCR for the virulence factors and markers *vt1*, *vt2*, *vt2c*, *vt2d*, *eae*, *ehxA*, *espP*, *saa*, *etp*, *katP*, and *subA* and by antibiotic resistance profiles. The PCR, conducted with the use of previously described primers (12–14,21–23) under optimized conditions, was performed in an Eppendorf Mastercycler (Brinkman Instruments, Westbury, New York, USA) with 25-μL reaction mixtures (2.5 μL of DNA, 2.5 μL of 10× PCR buffer, 1.5 or 2 mM $MgCl_2$, 200 μM of each deoxynucleoside triphosphate, and 2 U of *Taq* DNA polymerase). Strain EDL933 (*E. coli* O157:H7) was used as a positive control for PCR detection of *vt1*, *vt2*, *eae*, and plasmid genes including *ehxA*, *espP*, *katP*, and *etpD*. Strains E32511 (*E. coli* O157:NM) and B2F1 (*E. coli* O91:H21) were used as positive controls for *vt2c* and *vt2d*, respectively (22). A strain of O113:H21 VTEC isolated in this study was used as a positive control for *saa* and *subA*.

Antimicrobial susceptibility testing

Three to five colonies of each isolated VTEC serotype were tested for resistance to 11 antimicrobials by the disk diffusion method, as described by the Clinical and Laboratory Standards Institute (CLSI) (24). The panel of antimicrobials consisted of ampicillin (10 μg), amoxicillin/clavulanic acid (20/10 μg), nalidixic acid (30 μg), gentamicin (10 μg), kanamycin (30 μg), amikacin (30 μg), trimethoprim/sulfamethoxazole (1.25/23.75 μg), enrofloxacin (5 μg), spectimomycin (100 μg), tetracycline (30 μg), and chloramphenicol (30 μg). Antibiotic disks (BBL Sensi-Disk) were obtained from Becton, Dickinson and Company, Franklin Lakes, New Jersey, USA. The reference strain was *E. coli* ATCC 25922. Isolates were classified as susceptible, intermediate or resistant to each antimicrobial agent, and the intermediate readings were assigned to the resistant category. The chi-squared test was used to determine if the frequency of antibiotic resistance among the isolated VTEC serotypes was associated with their propensity to infect humans.

Results

Detection, isolation, and serotyping of VTEC

The prevalence of VTEC in the 500 samples, based on detection of VTs by the VT-ELISA in 51 samples and detection of *vt* genes by PCR in 31 samples, was 10.2% (95% CI, 7.8% to 13.2%) and 6.2% (95% CI, 4.4% to 8.7%), respectively. Of the 25 samples collected on

Table I. Characteristics of 24 VTEC serotypes isolated from rectal feces of cattle from an Ontario abattoir

	Genes												
Serotype[a]	*vt1*	*vt2*	*vt2c*	*vt2d*	*vt1* + *vt2*	*eae*	*ehxA*	*espP*	*saa*	*katP*	*etp*	*subA*	Antimicrobial resistance profile
02:H29	−	+	+	+	−	−	−	−	−	−	−	−	K, T/S, TE
015:H27	−	+	+	−	−	−	−	−	−	−	−	−	None
028:H25	−	+	+	−	−	−	+	+	+	−	−	+	None
028ac:H25	−	+	+	−	−	−	+	+	+	−	−	+	C
088:H25	−	+	−	−	−	−	+	−	+	−	−	+	None
089:H18	−	−	−	−	+	−	+	+	+	−	−	−	None
089:H21	−	−	−	−	+	−	+	−	+	−	−	+	None
091:H21	−	−	+	−	+	−	+	−	+	−	−	−	None
0103:H2	+	−	−	−	−	+	+	−	−	−	−	−	T/S
0103:H7	+	−	−	−	−	−	+	−	+	−	−	+	None
0113:H4	−	−	+	+	+	−	−	−	−	−	−	−	AM, T/S, TE
0113:H21	−	+	+	+	−	−	−	+	+	−	−	+	K, T/S, TE
0119:H16	−	+	−	−	−	−	−	−	−	−	−	−	AM, T/S, TE
0121:H7	+	−	−	−	−	−	−	−	−	−	−	−	None
0136:H12	+	−	−	−	−	−	−	−	−	−	−	−	None
0138:H38	−	−	−	−	+	−	+	+	+	−	−	+	None
0139:H19	−	−	−	−	+	−	+	+	+	−	−	+	None
0153:H19	+	−	−	−	−	+	+	+	−	−	−	−	None
0153:H25	−	−	−	−	+	−	+	+	+	−	−	+	T/S, TE, C
0153:H?	−	−	−	−	+	−	+	+	+	−	−	+	None
0163:H19	−	−	+	−	+	−	+	+	+	−	−	+	None
0168:NM	−	+	+	−	−	−	−	−	−	−	−	+	T/S, TE
0?:H16	−	+	−	−	−	−	−	−	−	−	−	+	AM, T/S, TE
0?:H28	−	−	−	−	+	−	+	+	+	−	−	−	None
Total	5	9	9	3	10	2	15	11	14	0	0	13	

K — kanamycin; T/S — trimethoprim/sulfamethoxazole; TE — tetracycline; C — chloramphenicol; AM — ampicillin.

[a] Serotypes in boldface and underlined text have been associated with severe disease in humans (hemorrhagic colitis, hemolytic-uremic syndrome, or both). Those in underlined text have been associated with diarrhea in humans.

each of the 20 sampling days, the numbers of ELISA-positive and PCR-positive samples ranged from 0 to 6 [mean = 2.6; standard deviation *(s)* +/− 1.8], and 0 to 4 (mean = 1.6; *s* +/− 1.1), respectively. The number of PCR-positive samples each week was always the same as or lower than the number of ELISA-positive samples. There was substantial agreement between the ELISA and PCR results: the kappa value was 0.63. Only 4 of the 31 PCR-positive samples (13%) were negative by ELISA, and VTEC were isolated from 27 (53%) of the 51 ELISA-positive samples. The 27 VTEC isolates belonged to 24 O:H serotypes, which were assigned to 17 O serogroups and 13 H types (Table I). No O157:H7 VTEC was isolated. Twelve (50%) of the serotypes have previously been associated with disease in humans: 7 of the serotypes (O2:H29, O91:H21, O103:H2, O113:H21, O153:H25, O163:H19, and O168:NM) have been associated with severe disease including hemorrhagic colitis and HUS, and the other 5 (O15:H27, O28ac:H25, O113:H4, O?:H16, and O?:H28) with diarrhea only (4,25). Four of the serotypes (O28:H25, O89:H18, O89:H21, and O138:H38) have not previously been reported as VTEC.

Detection of VTEC virulence factors

Among the 24 isolated VTEC serotypes, VT typing and subtyping by PCR revealed that 5 (21%) serotypes had only *vt1*, 3 (13%) had only *vt2*, and 7 (29%) had only *vt1* + *vt2* (Table I). Other toxin genotypes were *vt2* + *vt2*c in 4 (17%) serotypes, *vt2* + *vt2c* + *vt2d* in 2 (8%) serotypes, *vt1* + *vt2* + *2c* in 2 (8%) serotypes, and *vt1* + *vt2* + *vt2c* + *vt2d* in 1 (4%) serotype. Overall, 19 (79%) serotypes had *vt2* either alone or with *vt1* and/or *vt2* variants. Among the 7 serotypes that have been associated with severe disease in humans, 6 had *vt2* with or without other *vt* genes. The only 2 isolates positive for *eae* possessed the *vt1* gene. The distribution of plasmid-encoded putative virulence genes was as follows: *ehxA*, 63% (15/24); *espP*, 46% (11/24); *saa*, 58% (14/24); and *subA*, 54% (13/24); *katP* and *etpD* were not detected. Of the 13 *subA* positive serotypes, 12 (92%) were also *vt2*-positive. Among the 12 serotypes that have previously been reported as associated with disease in humans, 6 were *subA*-positive, and 4 of these 6 were those that have been incriminated in severe disease.

Antibiotic resistance patterns

Of the 24 serotypes, 9 (38%) were resistant to 1 or more of the antimicrobials. The highest frequencies of resistance were for trimethoprim/sulfamethoxazole (8/24) and tetracycline (7/24). Resistance was also observed to ampicillin (3/24), kanamycin (2/24), and chloramphenicol (2/24). Resistance to at least 1 antimicrobial

was significantly more frequent in serotypes associated with disease in humans (8/9) than in serotypes not known to cause disease in humans (1/9) ($P = 0.009$).

Discussion

Since production of VTs is the only common marker of all VTEC, detection of any VTEC in animal, food, or human samples typically relies on initial screening of fecal extracts or enrichment cultures, or both, for VTs or *vt* genes, followed by isolation of VTEC from positive samples (3,17,19,26). Historically, screening for VTEC relied initially on detection of VTs by the sensitive Vero cell cytotoxicity assay (VCA) and more recently on more efficient PCR assays for *vt* genes and ELISAs for VTs that have similar sensitivities and greater specificities compared with the VCA (17,26,27). Isolation of VTEC is then attempted by plating VT- or *vt*-positive samples or cultures and testing numerous colonies for VT production or *vt*, individually or by colony blot hybridization or immunoblotting. However, these isolation procedures are costly and laborious, and since the ratio of VTEC to non-VTEC in positive samples may be as low as 1:5000 (28), often only a portion of samples positive by sensitive and specific screening tests yields isolates. Consequently, VTEC prevalence is more reliably estimated by sensitive and specific screening tests than by isolation rates.

The prevalence rates for VTEC in this study (10.2% by VT-ELISA and 6.2% by PCR) were lower than the rates of 43.4% found with VCA and 46.5% (1002/2153) with PCR in Ontario dairy cattle (26), and 42% with the VCA in Alberta beef cattle (29) but higher than the 4% found with the VCA in beef cattle from Prince Edward Island (30). The wide range of values (2.1% to 70.1%) reported for beef cattle in many countries (6) may reflect true differences in prevalence but may also be influenced by differences in cattle populations, sampling strategies, sample handling, and laboratory methods. Because of marked differences in sampling procedures and laboratory methods, it is not possible to compare our data with those from other recent studies of the prevalence of VTEC in beef cattle in other regions of Canada.

Very few studies have reported the use of a VT-ELISA as a method of screening for VTEC in cattle fecal cultures. The prevalence in cattle obtained by VT-ELISA in this study is low compared with the 64% obtained by Randall et al (31) with the use of a different VT-ELISA that included mitomycin C during enrichment to enhance toxin production, which may have increased the assay's sensitivity (28). However, given the high sensitivity and specificity of the VT-ELISA used in our study in enrichment cultures of ground beef (16) and bovine and human fecal cultures (RPJ: unpublished data, 2006), such large differences may reflect true differences in prevalence. The prevalence obtained by PCR in this study (6.2%) was also low, and there was substantial agreement between the VT-ELISA and PCR results. The VT-ELISA did detect 24 positive samples that were not detected by PCR. These samples may be ones with strains that produced high levels of VT or had PCR inhibitors. One recent report (32) indicating that PCR is more sensitive after 6 h than after 24 h of enrichment suggests that testing the enrichment culture by PCR after 6 h incubation and by the VT-ELISA after overnight incubation might have yielded more consistent results. Another factor that may have contributed to the low prevalence by both VT-ELISA and PCR was the freezing of samples before processing (33).

The VTEC were isolated from 53% (27/51) of the VT-ELISA-positive samples by the colony immunoblot method, chosen because of its high efficacy and efficiency (17,28) and its ability to detect VTEC at ratios of up to 1:5000 among non-VTEC (28). Though slightly higher than the isolation rates of 49% obtained by colony hybridization from *vt*-PCR-positive fecal cultures (34) and 41.9% obtained by testing colonies and colony sweeps for VT by the VCA (23), the rate of isolation was lower than expected from previous findings with the immunoblot technique (17,28). Perhaps this difference reflects lower numbers of VTEC in the original samples or a reduction in numbers resulting from freezing before processing.

Half of the 24 VTEC serotypes that were recovered have been implicated in sporadic disease, including diarrhea, hemorrhagic colitis, and HUS, in humans (4). However, isolates belonging to serogroups O26, O45, O111, O145, and O157, which are frequently incriminated in severe disease in humans (3,9) were not recovered. The absence of O157 VTEC most probably reflects the use of a technique that detected all serotypes without selection and concentration of VTEC O157, as well as low numbers in the samples at the time of processing. Overall, the isolated serotypes and their virulence genotypes were consistent with those in previous reports on VTEC in cattle (6,35–37). Only 2 isolated serotypes were *eae*-positive, consistent with earlier observations that *eae* is infrequent in bovine VTEC (35). One of these *eae*-positive serotypes, O103:H2, is frequently associated with sporadic severe disease in humans; it carried the virulence factor profile (*vt1*, *eae*, and *ehxA*) typical of this serotype (9). Many of the serotypes (19/24) were *eae*-negative and *vt2*-positive, and these included 11 of the 12 isolated serotypes previously associated with disease in humans. Epidemiologic data suggest that humans infected with VTEC carrying *vt2* have a higher risk of severe disease, including HUS (7,8). Ritchie et al (38) compared production of VT2 with that of VT1 after mitomycin C induction and observed that the *vt2* gene was more responsive to induction. Greater inducibility could contribute to higher concentrations of toxin in the intestine of patients infected with *vt2*-positive organisms. The low proportion of *vt2d*-positive serotypes in our study is consistent with the results of a study by Bertin et al (37). Because VT2d has low toxicity for Vero cells (39) and has never been associated with HUS, *vt2d*-positive strains may be less virulent for humans (37).

The plasmid-encoded virulence markers *ehxA*, *espP*, and *saa* were present in a high proportion of VTEC isolates in this study, whereas *etpD* and *katP* were not present in any isolates. Jenkins et al (40) also reported a high proportion of *ehxA*- and *saa*-positive isolates among bovine VTEC and noted that the *saa* gene was frequent in isolates that were *eae*-negative and *ehxA*-positive. They suggested that Saa may play the role of an adhesin among *eae*-negative serotypes, facilitating their attachment to and colonization of the bovine intestine. Data are scarce on the occurrence of *katP*, *espP*, and *etpD* among bovine VTEC, possibly because of the lack of a consistent association between these plasmid determinants and disease in humans.

Of particular interest in this study was the presence of the *subA* gene in 54% of the isolated bovine VTEC. The serotypes in which *subA* was present were all *eae*-negative and mostly positive for *saa*, *ehxA*, *espP*, and *vt2*. The gene *subA* is a marker for SubAB, a

subtilase cytotoxin originally detected in VTEC O113:H21 strain 98NK, which was responsible for an outbreak of HUS in Australia (16). This 35-kilodalton AB5 toxin, a subtilase serine protease that binds GM2 ganglioside (16), is verocytotoxic and lethal to mice, causing large-scale microthrombi and necrosis in kidney, liver, and brain tissues (16). A Japanese research group recently reported that SubAB inhibits protein synthesis and induces vacuolation in Vero cells (41). The same researchers have identified $\alpha_2\beta_1$ integrin on the Vero cell surface as a potential receptor for SubAB (42). Owing to the lack of epidemiologic data on the presence of *subA* in VTEC strains, the significance of its presence in a high proportion of *vt2*-positive and *eae*-negative bovine isolates, and especially those isolates that have been associated with disease in humans, is still unclear. We suggest that the presence of *subA* in bovine VTEC serotypes may enhance their capacity to cause disease in humans, but this needs further investigation.

There was a high rate of resistance to trimethoprim/sulfamethoxazole and tetracycline and low rates of resistance to ampicillin, kanamycin, and chloramphenicol in 9 of the 24 VTEC isolates. Other studies have also recorded high rates of resistance, especially to tetracycline and sulfonamides, among VTEC of bovine and human origins (43). Seven of the nine resistant isolates, belonged to serotypes that have been incriminated in disease in humans (4). Antibiotic resistance was more prevalent among strains associated with disease in humans than among strains that have not been so implicated. Although antibiotic therapy for VTEC infections in humans remains controversial, the occurrence in cattle of antimicrobial-resistant serotypes that have been associated with disease in humans indicates that there is a risk of selection of these serotypes when antibiotics are used in cattle.

In summary, the VTEC carriage rate in this study was at the low end of the wide range of previously reported prevalence rates for beef cattle. The isolated VTEC belonged to 24 serotypes, were mostly *eae*-negative, and carried *vt2* alone or with other *vt* genes. The most prevalent plasmid-encoded potential virulence genes were *ehxA*, *saa*, and *subA*. The VTEC that were serotyped were all non-O157, and 50% were serotypes that have been implicated in disease in humans. Resistance to 1 or more antimicrobials was detected in 38% of the VTEC isolates. This study confirms that Ontario cattle remain an important reservoir of VTEC.

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