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Distribution and characteristics of verocytotoxigenic *Escherichia coli* isolated from Ontario dairy cattle

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SUMMARY

Faecal swabs obtained from a random sample of 1131 cows and 659 calves on 100 southern Ontario dairy farms were examined for verocytotoxigenic *Escherichia coli* (VTEC) using a Vero cell assay. Five isolates from each positive culture were tested similarly. Positive colonies were examined with DNA probes for Shiga-like toxin I (SLT-I) and SLT-II sequences. Probe-negative colonies were tested for neutralization of verocytotoxicity using anti-SLT-I and anti-SLT-IIv antisera. Colonies showing no neutralization response were examined in a polymerase chain reaction procedure. Colonies positive by any test were confirmed to be *E. coli* biochemically, serotyped, biotyped and tested for antimicrobial resistance. Faecal culture supernatants which were positive in the Vero cell assay, but culture negative, were examined using the neutralization assay. Animals were classified positive by faecal culture supernatant or by positive VTEC isolate. The prevalence rates of VTEC infection in cows and calves were estimated to be 9·5 and 24·7%, respectively. The proportion of animals infected on each farm ranged from 0 to 60% for cows and 0 to 100% for calves. Of 206 VTEC isolates identified, few were of serotypes which have been isolated from humans and none were *E. coli* O 157 : H7.

INTRODUCTION

Infection of humans with verocytotoxigenic *Escherichia coli* (VTEC) has been linked to a spectrum of illness which includes watery diarrhoea, bloody diarrhoea and the haemolytic uraemic syndrome (HUS) [1, 2]. In outbreaks of diarrhoeal disease and HUS, infection with VTEC has been associated with consumption of meats, and in particular beef [1, 3]. VTEC infection accompanied by diarrhoeal disease has also been reported in children following the consumption of unpasteurized milk [4, 5]. These findings have prompted many workers to suggest

that VTEC infection may be food-borne. Indeed, several surveys have shown that contamination of beef and other meats with VTEC is a common occurrence [6–8]. VTEC were also identified in a survey of milk filters from Ontario dairy farms [9].

Identification of the source of contamination of foods with VTEC is important to the understanding of the epidemiology of human VTEC infection and ultimately to devising strategies for its control. Foods of animal origin may be contaminated with VTEC by contact with faeces of animals harbouring VTEC within their intestinal tracts. In particular, the frequent association between VTEC infection and the consumption of beef or dairy products suggests that dairy cattle, an important source of both meat and milk for human consumption, might be an important reservoir for human food-borne VTEC infection [4, 5, 10, 11].

One means of assessing the potential importance of cattle as a reservoir for human VTEC infection is to estimate the prevalence of VTEC infection in the bovine population. To ensure their validity, such prevalence estimates must be based on a formal random sample from a well-defined population [12]. Although several preliminary surveys of VTEC infection in cattle have been published [13–17], few of these have used a formal random sampling strategy. In a study of cattle from an Ontario abattoir in which random sampling was used [18], Clarke and co-workers found 19·5% of cull dairy cows to be infected with VTEC strains. However, a sample of cull cattle at slaughter, even if randomly selected, may not reflect the rate of infection in the general dairy cattle population. For example, the rate of infection among cows at slaughter may increase as a result of cross-infection caused by mixing of animals from different sources. Also, cull dairy cattle are, on the whole, considerably older than the general dairy cow population.

Another important feature of surveys of this type is the selection of the organizational unit of interest – the farm or the individual animal. Most surveys of bovine VTEC infection to date have expressed the prevalence of infection as the proportion of infected individuals in the population. This is valuable since it provides a means of assessing the risk to humans of being exposed to a given individual animal randomly selected from among all animals in the population. Equally important from a public health standpoint, however, is an estimate of the risk associated with being exposed to a given farm selected at random from among all farms in the population, that is, a measure of farm-level prevalence of infection. This aspect of the epidemiology of VTEC infection has so far received little study.

Of course, the production of VT by an *E. coli* isolate does not constitute proof of pathogenicity. Although several VTEC serotypes have been isolated from humans, strong evidence for pathogenicity exists only for relatively few, such as O 157.H7. Thus the distribution of individual VTEC serotypes in the bovine population is also of potential public health significance.

The objectives of the current study were to describe the distribution of bovine VTEC infection at the herd and individual animal levels in a well-defined population of dairy cattle in southern Ontario, and to characterize individual VTEC isolates obtained from this population according to serotype.

MATERIALS AND METHODS

Sampling strategy

The number of herds required for study was estimated by first calculating the sample size needed to estimate the proportion of VTEC-infected farms within reasonable confidence limits, and then calculating sample sizes required to test simple univariate hypotheses at the herd level [12]. These calculations were then evaluated in light of time and cost limitations resulting in a decision to select 100 farms in total.

Due to economic and time constraints it was decided to limit the population under study to dairy farms within four contiguous counties (Wellington, Halton, Peel and Hamilton-Wentworth) within a 60 mile radius of the City of Guelph. In 1988 a list was obtained of all milk producers registered with the Ontario Milk Marketing Board in that year in these four countries. (Ontario law requires that anyone in the Province producing cow's milk for sale must be registered with this Marketing Board.) The names of all producers were then ordered randomly using a computerized random number generator. The first producer in this random sequence was contacted by telephone and asked to participate in the study. In order to obtain 100 producers willing to participate, it was necessary to contact 121 producers in this manner.

Farm visits

All 100 farms were visited once between March and October of 1988. On farms with 40 or more dairy cows milking on the day of the visit, a formal random sample of 25% of these cows was selected for testing. On farms with fewer than 40 cows milking on that day, a random sample of 10 was selected. A single faecal swab was obtained from each of the cows selected and from all dairy calves 3 months of age or younger on each farm. Faecal swabs were placed in transport media on ice and were taken to the laboratory for immediate processing.

Preparation and storage of samples

Testing of faecal samples for the presence of VT and individual VTEC isolates was undertaken in a multi-stage manner (Figs. 1 and 2). Swabs were placed in 5 ml of MacConkey broth and incubated overnight at 37 °C. One ml of each broth culture was then added to an equal volume of 50% glycerol and stored in individual vials at −70 °C for use in subsequent studies. The remaining broth culture was stored in a refrigerator for 1 week.

Screening of crude faecal cultures for verocytotoxicity

Crude faecal cultures were tested for verocytotoxicity using a screening assay described by Clarke and co-workers [9] with the following modifications. 100 μl of MacConkey broth culture was added to 1 ml of brain heart infusion broth (BHIB) in a sterile microcentrifuge tube. BHIB cultures were incubated overnight at 37 °C and then centrifuged at 12000 g for 10 min. Fifty μl of supernatant were added to duplicate wells in a 96-well flat-bottomed microtitre plate containing 200 μl of Eagles minimal essential media (EMEM, Gibco) in each well. One hundred μl of a

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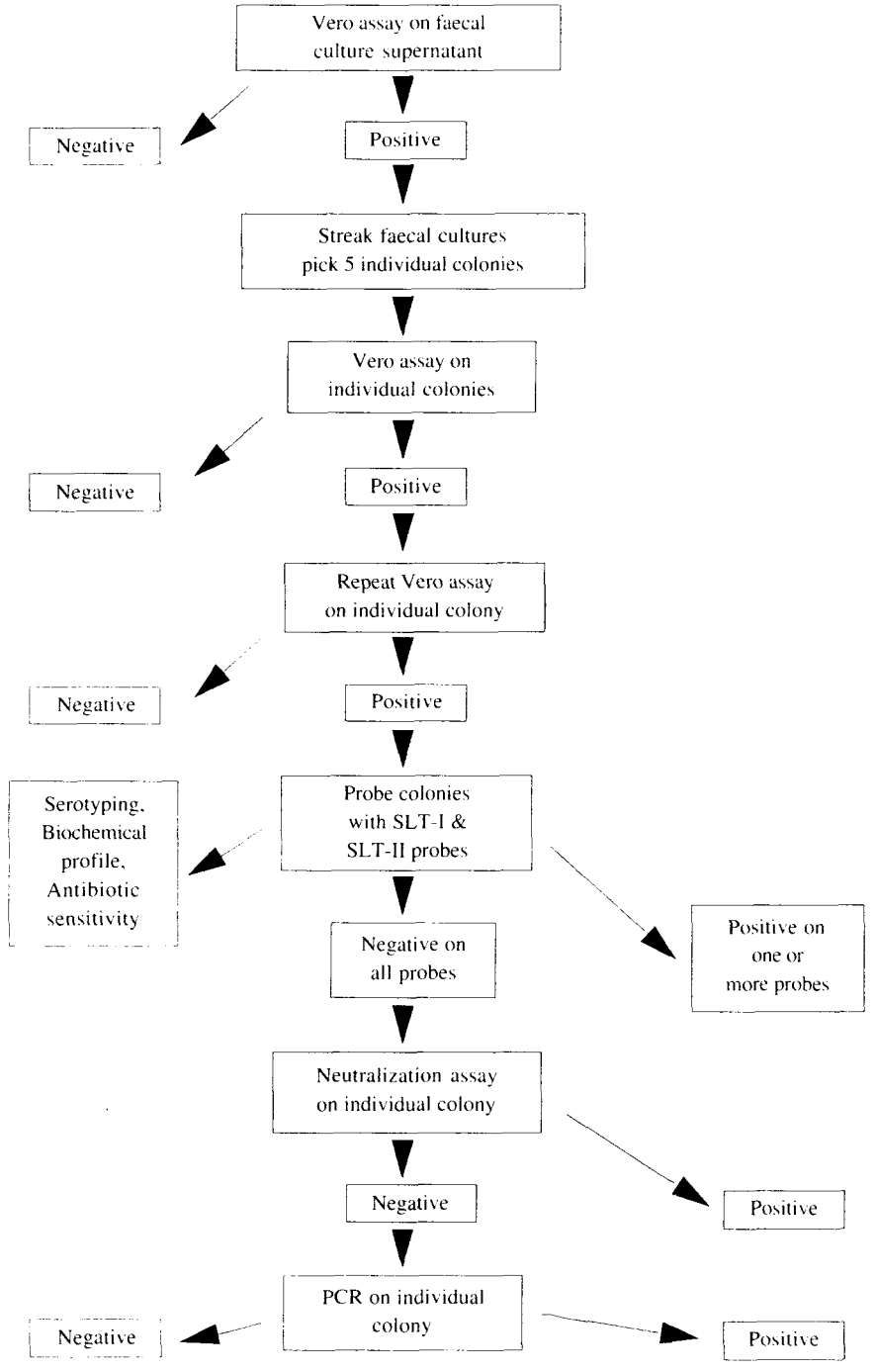

Fig. 1. Flow chart of procedures used to identify and characterize individual VTEC isolates.

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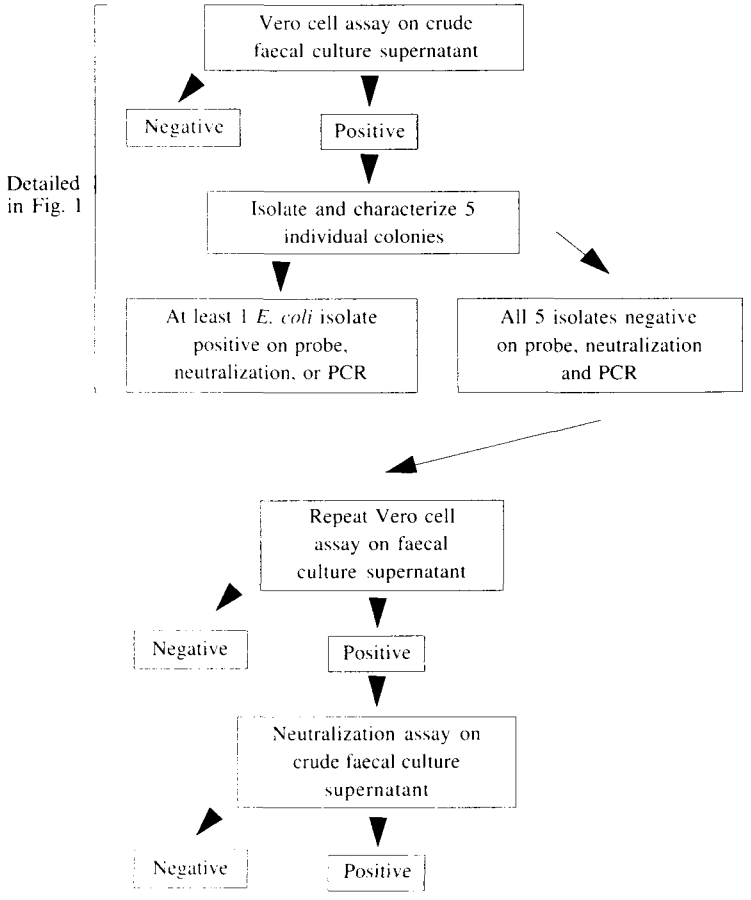

Fig. 2. Flow chart of procedures used to identify VT-positive animals.

suspension of Vero cells (containing approximately 4×10^5 cells/ml) were then added to each well. The plates were incubated at 37 °C in a 5% CO_2 incubator for 48 h. fixed with 10% formalin. stained with crystal violet. washed and then examined under an inverted microscope to estimate the degree of destruction of the Vero cell monolayer. Wells having at least 50% destruction of the monolayer. as compared to a standard control plate. were considered positive.

Screening of isolates for verocytotoxicity

In order to isolate and characterize individual VT-producing *E. coli* strains from positive animals. the original MacConkey broth cultures positive in this screening assay were inoculated onto MacConkey agar plates. These plates were then incubated at 37 °C overnight. Five individual lactose-fermenting colonies were picked from each plate. and inoculated into microcentrifuge tubes containing 1 ml of BHIB. Each isolate was then screened for VT production as described for the crude faecal culture supernatants. For colonies positive on this assay the process was repeated. Cultures of all colonies positive on one screening assay were stored at −70 °C in 50% glycerol.

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Biochemical profile, antimicrobial resistance pattern and serotype of isolates

Colonies positive on two consecutive screening assays were then characterized according to a series of further tests. In each case, testing was performed on material obtained from the frozen cultures. Isolates were confirmed to be *E. coli* and biotyped using the Repliscan system (Cathra International Inc., St Paul, Minnesota). The following biochemical reactions were determined: bile tolerance; growth on colistin/nalidixic acid agar, cetrimide/kanamycin agar, and colistin agar; acidification of mannitol, 10% lactose, cellobiose, glucose, arabinose, sorbitol, sucrose, rhamnose and inositol; decarboxylation of lysine and ornithne; utilization of citrate and malonate; hydrolysis of esculin and arginine; production of hydrogen sulphide and indole.

Antibiotic resistance patterns of *E. coli* isolates were also determined using the Repliscan system. Repliplate media (Cathra Systems) containing drugs at the following concentrations were used: gentamicin and tetracycline (4 μg/ml); ampicillin, chloramphenicol and neomycin (8 μg/ml); kanamycin and spectinomycin (16 μg/ml); sulphisoxazole (256 μg/ml); trimethoprim/sulphamethoxazole (2/38 μg/ml). In order to test for resistance to ampicillin/clavulanic acid, plates were prepared by adding amoxicillin trihydrate (Ayerst Laboratories, Montreal, Canada) dissolved in phosphate buffer (pH 7·0), and clavulanic acid (Beecham Laboratories, Pointe Claire, Quebec, Canada) dissolved in deionized water to Mueller–Hinton agar (BBL Microbiological systems, Cockeysville, MD, USA) at a final concentration of 8 μg/ml amoxicillin and 4 μg/ml clavulanic acid.

Isolates were serotyped at the Pennsylvania State University *E. coli* Reference Center.

DNA probing of isolates

Presence of VT genes in *E. coli* isolates was verified by using two oligonucleotide probes directed against the genes coding for SLT-I and SLT-II. The probes were designed on the basis of published nucleotide sequences for these two toxins. Differences in the B subunit coding region of the SLT-I and SLT-II genes were identified using the Eugene program developed at the Baylor College of Medicine. The nucleic acid sequence for each oligomer was verified by comparing each sequence with the entire GenBank data base. Oligonucleotides were synthesized using an Applied Biosystems synthesizer by the automated phosphoramidite method. Oligonucleotides were then labelled with T4 kinase (NBL) and ^{32}PdATP (Amersham). NENsorb columns (New England Nuclear) were used to separate the radiolabelled nucleotides from unincorporated ^{32}P.

A colony blot procedure was used to examine each isolate for hybridization with each of the two probes. The procedure was as follows: isolates were grown overnight in BHIB at 37 °C, and transferred to duplicate wells in a 96-well microtitre plate. Positive controls of *E. coli* strains known to produce SLT-I (strain H30), SLT-II (strain 933W) and VT2 (strain E32511) were included on each plate.

Isolates were transferred from the plates to wet nylon filters (Amersham) using a multipoint inoculator. Filters were laid on a 150 mm MacConkey agar plate and incubated at 37 °C for 4 h. They were then placed colony side up on blotting paper saturated first with 0·5 M-NaOH and 1·5 M-HCl followed by 1·5 M-NaCl and 1·0 M

Table 1. *Neutralization of verocytotoxins with homologous and heterologous antisera (from MacLeod and Gyles, 1990, with permission)*

		Titre* Antigen			
		SLT-IIv	SLT-II	VT2	SLT-I
Antiserum	SLT-IIv	20,480	1,280	2,560	—†
	SLT-I	—	—	—	10,240

* Titre expressed as reciprocal of highest dilution of a 50 μl volume of serum which protected Vero cells from 10 CD50 of SLT or VT.

† No neutralization.

Tris-Hcl, pH 7, (2 × 7 min). Filters were air-dried and the DNA cross-linked by inversion of the filter over a transilluminator (302 nm) for 4 min. This was followed by agitation in pre-hybridization solution with 50% formamide overnight at 42 °C. Hybridization (using 10^7 c.p.m. per ml of hybridization solution) was carried out for 16 h at 42 °C. Filters were then washed at 65 °C for 1 h in 2 × SSC, 0·1 % SDS, and then for 1 h in 0·1 × SSC, 0·5 % SDS. They were then air-dried and used for autoradiography with Kodak X-AR film.

Neutralization assay of isolates

Isolates which failed to hybridize with either of the two probes were tested by a neutralization assay. The method was similar to that described by Scotland [19], with minor modifications. The antiserum consisted of a combination of equal volumes of two separate antisera; one against SLT-I and the other against SLT-IIv [20, 21]. Titres of each antiserum are shown in Table 1.

For the assays a loop from the frozen MacConkey culture was inoculated into BHIB in a microfuge tube, cultured and centrifuged as described for crude supernatants. Individual culture supernatants were added to microtitre plates as above, this time using four replicates of each sample per plate rather than two. For each sample, 50 μl of a 1:50 dilution of combined antiserum were added to two of these wells, and to the other two, 50 μl of phosphate buffered saline (PBS) were added. Plates were incubated at 37 °C for 1 h and then overnight at 5 °C. Vero cells were added and the plates processed as described previously. A positive neutralization response consisted of clear reduction in Vero cell death in wells containing antiserum compared to corresponding wells containing PBS.

For some samples, there was complete destruction of the monolayers in both antibody and PBS-containing wells, suggesting that these samples might possess very high VT titres. For these samples, the neutralization assay was repeated using several serial fivefold dilutions of culture supernatant. Dilutions were performed on each sample, either to demonstrate a positive neutralization response (as defined above), or to dilute the supernatant to the extent that no significant destruction of the monolayer occurred in the PBS-containing wells.

Detection of VT genes by polymerase chain reaction

Colonies which failed to demonstrate a positive neutralization response were tested using a polymerase chain reaction (PCR) procedure. A pair of synthetic oligonucleotide primers targeting conserved regions of gene sequences for SLT-I,

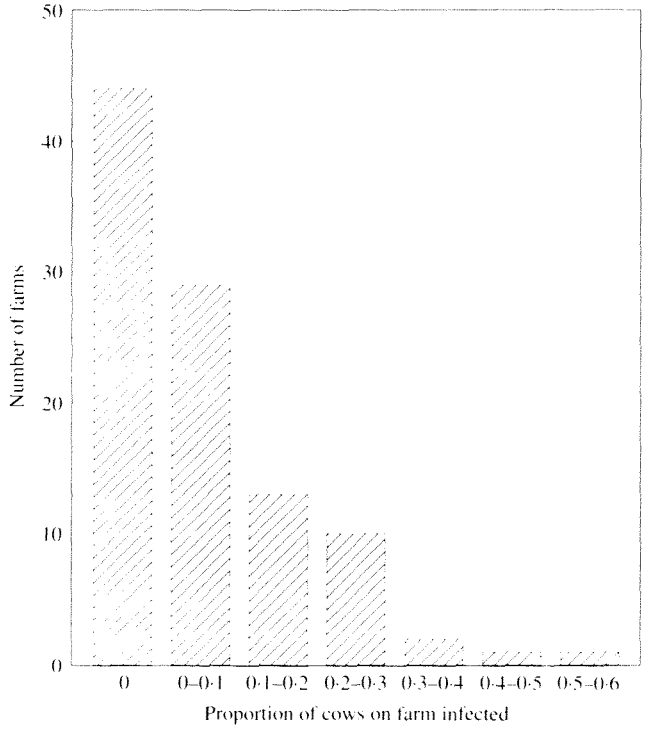

Fig. 3. Frequency distribution of herd-level VTEC infection in milking cows. Each bar represents the number of farms with proportion of infected cows greater than lower bound indicated, and less than or equal to upper bound.

VT2, and SLT-IIv were used (designed and supplied by Cangene Corporation, Toronto, Ontario). The PCR reaction was performed using the PCR Core Reagents kit (Perkin Elmer Cetus, Norwalk, Conn). Reaction mixtures were amplified through 35 cycles in a DNA Thermal Cycler (Perkin Elmer Cetus) and the amplified product was visualized using standard agarose gel electrophoresis.

Neutralization assay on crude faecal cultures

Failure to identify a VTEC isolate in faecal cultures demonstrating verocytotoxicity using the methods described suggests that VTEC may be present in the sample, but at relatively low numbers. To increase the sensitivity of detection of VTEC-infected animals, frozen faecal cultures from animals positive using the initial VT screening assay on faecal culture supernatants, and from which an individual VTEC isolate was not obtained, were subjected to the screening assay once again. Cultures positive on this second VT screening assay were then examined using the neutralization procedure, as described for individual colonies in order to determine whether Vero cell death was due to the presence of verocytotoxins.

Classification of infection status

Individual *E. coli* isolates were classified positive if they hybridized with either of the two DNA probes, if they demonstrated a positive neutralization response, or if they were positive by gene amplification in the PCR procedure (Fig. 1). Animals were classified positive if their crude faecal cultures demonstrated a

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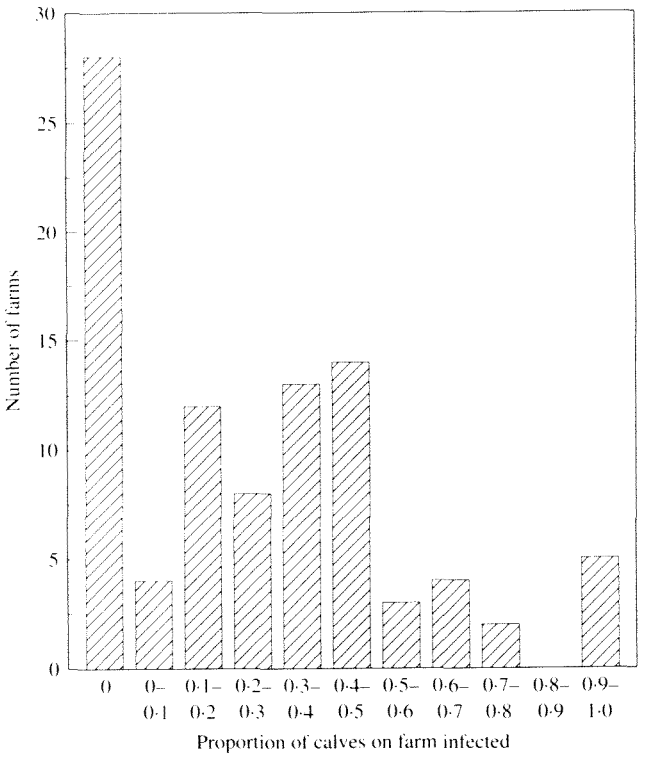

Fig. 4. Frequency distribution of herd-level VTEC infection in calves less than 3 months of age. Each bar represents the number of farms with proportion of infected calves greater than lower bound indicated, and less than or equal to upper bound.

positive neutralization response, or if a positive individual *E. coli* colony had been isolated from their faeces (Fig. 2).

Data processing

All data were entered into a computer data base program (Dbase III plus, Ashton Tate, Torrance, CA) by a technical assistant and checked against the original for validity by the principal investigator. In addition, a series of logical checks was performed using programs designed to identify entries outside of an appropriate range.

Statistical analysis

Intra-herd correlation coefficients for infection status were calculated separately for cows and calves. These were then used to adjust standard errors for estimates of the population prevelance of infection for cows and calves separately, and then for the estimated difference in infection prevalence between the two groups [22]. Standard errors were then used to construct 95% confidence intervals for each prevalence using the normal approximation to the binomial distribution [22].

RESULTS

Distribution of VT positive animals

The prevalence of infection was 9·5% for cows and 24·7% for calves. The corresponding 95% confidence intervals were (7·1, 11·8) and (20·2, 29·3).

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Table 2. *Biotypes of VTEC isolated from farms and individual animals*

Biochemical reactions*								Number of		
Lys	Orn	Esc	Arg	Sor	Suc	Rha	Ind	Isolates	Animals†	Farms‡
+	−	−	−	+	−	+	+	69	43	32
+	+	−	−	+	−	+	+	56	38	30
+	−	−	−	+	−	−	+	27	17	12
+	+	−	−	+	+	+	+	18	14	10
+	+	−	−	+	+	−	+	8	7	6
−	−	−	−	+	−	+	+	6	3	2
+	+	−	−	+	+	−	+	5	5	5
+	−	−	−	+	+	+	+	4	3	3
−	+	−	−	+	+	+	+	2	2	2
+	−	−	−	−	−	−	+	1	1	1
+	−	−	−	−	−	+	+	1	1	1
+	−	−	−	+	−	−	−	1	1	1
+	−	−	−	+	−	+	−	1	1	1
+	−	−	−	+	+	−	+	1	1	1
+	−	+	−	+	−	−	+	1	1	1
+	−	+	−	+	−	+	+	1	1	1
+	+	−	−	−	+	+	+	1	1	1
+	+	−	−	+	+	−	−	1	1	1
+	+	−	+	+	−	+	+	1	1	1
+	+	+	−	+	−	+	+	1	1	1

* Lys, Decarboxylation of lysine; Orn, decarboxylation of ornithine; Esc, hydrolysis of esculin; Arg, hydrolysis of arginine; Sor, acidification of sorbitol; Suc, acidification of sucrose; Rha, acidification of rhamnose; Ind, production of indole.

† Number of animals from which VTEC with the indicated biotype were isolated.

‡ Number of farms from which VTEC with the indicated biotype were isolated.

respectively. The difference in the prevalence of infection between cows and calves was statistically significant; the 95% confidence interval for this difference was (10·2, 20·3). The intra-herd correlation coefficient for infection of cows was 0·08, and that for calves was 0·14.

The distribution of the estimates of proportion of cows infected on each farm is shown in Figure 3 (mean proportion of cows infected 0·097, standard deviation 0·12). The corresponding data for calves are shown in Fig. 4 (mean 0·29, standard deviation 0·28). Many farms had either no infected cows or no infected calves resulting in a marked right skewness of the frequency distributions.

Distribution of VT-positive isolates

A total of 206 individual VT-producing *E. coli* isolates was identified in this study; 68 of these were from cows and 138 from calves. Thirty-six cows (3·2%) and 57 calves (8·6%) had at least one confirmed VTEC isolated from their faeces.

Biochemical profiles, serotyping, antimicrobial resistance patterns, and DNA probe hybridization

All individual isolates positive on two consecutive VT screening assays were confirmed to be *E. coli* on the basis of biochemical reactions. Results of biochemical tests for which there was variation among VTEC isolates are shown in Table 2. Results of DNA probe hybridization studies are shown in Table 3.

Tables 4 and 5 show antimicrobial resistance patterns of isolates separately for cows and calves. Twenty-eight (41%) of isolates obtained from cows and 101 (73%) of isolates from calves were resistant to at least one of the drugs tested. Forty-three cow and 37 calf isolates were sensitive to all drugs examined. Results of serotyping of VTEC isolates are shown in Table 6.

DISCUSSION

The results of this study suggest that VTEC infection is widespread among dairy cattle in the Province of Ontario. Furthermore, infection is apparently not evenly distributed within age groups or across all farms. The estimated proportion of cows infected on each farm ranged from 0 to 60% and for calves the range was from 0 to 100%. In addition, we found the prevelance of VTEC infection in calves to be significantly higher than in cows, a finding also reported recently by Wells and colleagues [17] in a survey of VTEC infection in Wisconsin dairy cattle. One can only speculate as to reasons for this apparent age effect. Possible explanations might be a relative lack of immunity to VTEC infection in young animals, physiological or morphological characteristics of the gastrointestinal tract of calves which promote colonization by VTEC, or aspects of calf management which increase the opportunity for exposure to these organisms.

Comparisons between results of prevalence studies should be made with caution, but are useful provided that one is aware that differences between studies can arise as the result of such factors as the choice of the target population, differences in sampling strategies, and variations in the microbiological test methodologies employed. A study, comparable in many respects to the current one, was conducted by Clarke and co-workers [18] who examined faecal specimens from 600 cattle randomly selected from an Ontario abattoir. Faecal samples were tested in a Vero cell screening assay similar to that used in the present report. They identified VT-positive isolates in samples from 21 (10·5%) of 200 beef cattle, 39 (19·5%) of 200 cull dairy cows, and 7 (3·5%) of 200 veal calves. The relatively low isolation rate in calves compared with the present study might be accounted for in part by the fact that veal calves in Ontario, as opposed to calves raised on dairy farms, are routinely treated with a broad range of antibiotics over much of their lives.

Several other authors have reported the results of surveys of VTEC infection in cattle. In the USA, Wells and colleagues [17] identified *E. coli* O 157.H7 in 7 of 317 heifers and calves from dairy farms in Washington State and from 10 of 262 dairy heifers and calves in Wisconsin. Non-O 157-VTEC were identified in 13 of 154 adult dairy cows and 32 of 168 dairy heifers and calves in the Wisconsin investigation. In Sri Lanka, Mohammad and co-workers [23] collected faecal samples from both diarrhoeic and non-diarrhoeic calves and tested a single *E. coli* isolate from each animal using a VT neutralization assay. They identified a positive VTEC isolate in 27% of diarrhoeic calves and 10% of non-diarrhoeic calves from the same herds. In a slaughterhouse survey in Sheffield, England [13], faecal samples from 2 of 207 randomly selected cattle were positive for *E. coli* O 157.H7. Positive samples were identified by inoculating faecal samples onto sorbitol MacConkey agar, and testing non-sorbitol fermenting *E. coli* for

Table 3. *Hybridization of DNA probes among VTEC isolates*

Probe		Number of		
SLT-I	SLT-II	Isolates	Animals*	Farms†
+	−	67	31	23
−	+	74	33	21
+	+	22	13	9
−	−	43	31	24

* Number of animals from which VTEC isolates with the indicated hybridization response were obtained.

† Number of farms from which VTEC isolates with the indicated hybridization response were obtained.

Table 4. *Antibiotic resistance patterns of VTEC isolated from cows*

Number of isolates	Antibiotic(s)*						
1	ap						
13	sp						
2	sp	te					
3	su	te					
1	su	te	te				
2	ap	sp	su	te			
1	km	sp	su	te			
1	km	nm	sp	su	te		
1	ap	au	km	nm	sx	te	
1	ap	au	ch	sp	su	sx	te

* ap. Ampicillin; au. augmentin; ch. chloramphenicol; km. kanamycin; nm. neomycin; sp. spectinomycin; su. sulphisoxazole; sx. trimethoprim/sulphamethoxazole; te. tetracycline.

agglutination with *E. coli* O 157 antiserum. Finally, in a recent survey of German cattle, Montenegro and co-workers [15] identified VTEC isolates in 17% of cows and 9% of bulls from unspecified sources using VT1 and VT2 gene probes.

The VTEC isolates identified in this study encompassed a range of biotypes. This is consistent with the report by Clarke and colleagues [18]. In contrast to Clarke's report, however, we found a large proportion of isolates from mature cows to be resistant to a variety of antimicrobials, despite the fact that similar test methodology was used in both studies. The reasons for this difference are not clear. In addition, we found a higher rate of antimicrobial resistance among isolates obtained from calves than for those obtained from cows. This is not surprising since calves in Ontario are more frequently treated with antimicrobials than are lactating dairy cows [24, 25].

Most of the isolates tested with DNA probes in the present study hybridized with either or both of the SLT-I and SLT-II probes. Similar results were reported by Montenegro and colleagues [15] as discussed above.

A large number of different VTEC serotypes was identified in this study. Many other authors have similarly reported finding a wide range of VTEC serotypes in the faeces of apparently healthy cattle [15–18, 23]. However, we isolated no *E. coli* of serotype O 157:H7. This finding is consistent with previous large surveys of VTEC in cattle, most of which have suggested that *E. coli* O 157:H7 is uncommon

Table 5. *Antibiotic resistance patterns of VTEC isolated from calves*

Number of isolates	Antibiotic(s)*							
2	ap							
11	sp							
2	su							
4	sx							
4	te							
1	ap	au						
1	ap	sp						
1	ap	te						
2	sp	sx						
1	sp	te						
15	su	te						
1	sx	te						
4	ap	sp	sx					
1	ap	su	te					
8	sp	su	te					
1	su	sx	te					
2	ap	km	nm	te				
6	ap	sp	su	te				
5	ch	sp	su	te				
1	km	nm	te	sp				
2	sp	su	sx	te				
3	ap	au	sp	su	te			
7	ap	ch	sp	su	te			
1	ap	km	nm	sp	te			
3	ap	km	nm	su	te			
1	ch	sp	su	sx	te			
1	gn	nm	sp	su	te			
5	km	nm	sp	su	te			
1	ap	ch	km	nm	su	sp		
1	ap	km	nm	sp	su	te		
1	ap	au	km	nm	su	sx	te	
1	ch	km	nm	sp	su	sx	te	
1	ap	ch	km	nm	sp	su	sx	te

* ap, Ampicillin; au, augmentin; ch, chloramphenicol; gn, gentamicin; km, kanamycin; nm, neomycin; sp, spectinomycin; su, sulphisoxazole; sx, trimethoprim/sulphamethoxazole; te, tetracycline.

in the bovine population. For example, Clarke and co-workers found *E. coli* O 157.H7 in 3 of 200 beef cows and 1 of 200 dairy cows [18]. Similarly, Montenegro and colleagues found *E. coli* O 157.H7 in 2 of 259 mature cattle [15] in Germany, while Chapman and co-workers found 2 *E. coli* O 157.H7 in a slaughterhouse survey of 207 cattle in Sheffield, England [13]. Mohammad and colleagues [23] found no O 157.H7 in their study of 273 calves.

Other authors have reported higher rates of O 157.H7 infection among cattle on farms epidemiologically linked either to outbreaks or sporadic cases of O 157.H7-associated illness in humans. For example, Wells and co-workers [17] examined faecal samples from cattle on two separate Wisconsin dairy farms where, in unrelated incidents, two young children had developed O 157.H7 infection and HUS after drinking raw milk. These investigators found *E. coli* O 157.H7 in 5·9% of 85 calves and heifers examined on these two farms. Similarly, following an

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Table 6. *Serotypes of VTEC isolated from individual animals and farms*

Serotype	Number of Isolates	Animals*	Farms†
O 2.H–	1	1	1
O 3c.H–‡	1	1	1
O 4.H7	1	2	2
O 4w.H7§	1		
O 4c.H16	1	2	2
O 4wc.H16	1		
O 4.NM	1	1	1
O 6.H–	1	1	1
O 8w.H8	2	2	2
O 8.H16	1	1	1
O 8w.NM	6	4	4
O 8w.H–	6	3	3
O 9w.NM	1	1	1
O 11.H–	2	2	2
O 15.NM	1	1	1
O 22.H1	1	1	1
O 22.H8	1	1	1
O 25.Hmult‖	1	1	1
O 26.NM	1	2	2
O 26w.NM	1		
O 32.H7	1	2	2
O 32w.H7	1		
O 32w.H16	1	1	1
O 32.NM	1	1	1
O 32w.H–	1	1	1
O 40.H–	1	1	1
O 43.H2/6/12¶	1	1	1
O 82w.NM	1	1	1
O 87/X3.NM	1	1	1
O 103.H2/6/12	1	1	1
O 103.H16	1	1	1
O 103.H39	2	2	2
O 103.H39w	1		
O 103.H–	2	2	2
O 103wH–	1		
O 103.Hmult	1	1	1
O 106.H–	2	2	1
O 109w/119w.H16w	1	1	1
O 111w.NM	1	1	1
O 113w.NM	1	1	1
O 117.H–	1	1	1
O 117.NM	1	1	1
O 121.H7	1	2	2
O 121.H7w	1		
O 121.NM	1	1	1
O 121.H–	2	2	2
O 146.NM	1	1	1
O 153w.H7	1	1	1
O 153w.H32	1	1	1
O 153.NM	3	4	3
O 153w.NM	3		
O 153.H–	1	2	2
O 153w.H–	1		

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Table 6. *Cont.*

Serotype	Number of Isolates	Number of Animals*	Number of Farms†
O 163.H−	1	1	1
O −.H2/3/6/8/12	1	1	1
O −.H2/6/12	1	1	1
O −.H6w	2	2	2
O −.H7	7	6	6
O −.H7w	1		
O −.H8	7	5	5
O −.H12	1	1	1
O −.H16	1	3	3
O −.H16w	2		
O −.H21	4	2	2
O −.H21w	1		
O −.H40	4	2	2
O −.NM	20	17	15
O −.H−	44	34	27
O −.Hmult	5	5	5
O X3.H7/21	1	1	1
O X3.H16	4	4	3
O X3.H21	2	2	2
O X3w.H21	2		
O X3.H39	1	1	1
O X3.NM	7	7	6
O X3w.NM	4		
O X3.H−	3	6	5
O X3w.H	4		
O X8w.H−	1	1	1
O mult.NH	2	2	2

* Number of animals from which VTEC with the indicated serotype were isolated.
† Number of farms from which VTEC with the indicated serotype were isolated.
‡ Lower-case 'c' indicates positive reaction at 121 °C only.
§ Lower-case 'w' indicates weak reaction.
‖ Multiple reactions.
¶ Strong reactions of equal titre.

outbreak of HUS and *E. coli* O 157.H7 infection in a group of schoolchildren that visited an Ontario dairy farm, Duncan and colleagues [4] found *E. coli* O 157.H7 in two young dairy animals on the farm in question. It would seem that farms linked to such incidents of human illness have a higher prevalence of bovine O 157.H7 infection than the general dairy cattle population.

Many serotypes of VTEC, other than O 157.H7, have been isolated from humans with diarrhoeal disease or HUS, and the list is growing steadily [10]. However for most of the serotypes identified in this study, isolation from humans has not, to our knowledge, been reported. Other investigators studying the distribution of VTEC in cattle [15, 18, 23] have likewise reported a high proportion of bovine VTEC isolates belonging to serotypes that have not been identified in people. This could be interpreted as evidence that not all VTEC in dairy cattle are pathogenic for humans, although it may be only a matter of time before many more of the serotypes of VTEC isolated from cattle are identified in cases of human illness.

In summary, our results suggest that VTEC infection is common in dairy cattle

in Ontario, and particularly in young calves. However, a number of VTEC serotypes harboured by these animals have not been isolated from humans. Most significantly, infection of dairy cattle with *E. coli* O 157 : H7 appears to be relatively rare in the Province of Ontario, at least in comparison to other VTEC serotypes. Beyond such generalizations, the public health significance of VTEC infection in cattle is difficult to assess accurately at this point. Further research is required to identify the factors which promote proliferation of VTEC at various points within the human food chain, to determine the number of organisms required to cause disease, and to clarify the relationship between VTEC serotype and pathogenicity.

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