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Molecular typing methods to investigate transmission of *Escherichia coli* O157:H7 from cattle to humans

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SUMMARY

The utility of phage typing, pulsed-field gel electrophoresis (PFGE), and plasmid profile analysis was compared, to differentiate between Canadian *Escherichia coli* O157:H7 strains of human ($n = 27$) and cattle ($n = 24$) origin. The diversity indices for phage typing, plasmid analysis and PFGE were 0·85, 0·69 and 0·93, respectively. PFGE and phage typing were also applied to study the role of direct transmission of *E. coli* O157:H7 from cattle to humans on isolates collected from two separate farm outbreaks. PFGE showed that more than one *E. coli* O157:H7 strain with varying PFGE DNA subtype profiles, may be responsible for an outbreak, and that more than one *E. coli* O157:H7 subtype may be circulating on a particular farm at any one time. To our knowledge, this is one of the first reports where PFGE typing was used to verify the direct transmission of *E. coli* O157:H7 from cattle to humans.

INTRODUCTION

Escherichia coli serotype O157:H7 is associated with a broad spectrum of illness including non-bloody diarrhoea, haemorrhagic colitis, haemolytic uraemic syndrome (HUS) and thrombotic thrombocytopaenic purpura [1–2]. Recent explosive outbreaks in Canada, United States, and Japan have augmented the public health importance of this foodborne pathogen [3–6]. Outbreaks of *E. coli* O157:H7 have been epidemiologically linked to the consumption of contaminated foods, and person-to-person transmission [1, 4, 7, 8]. Cattle are thought to be the reservoir for human infection [9–11]. There have been studies implicating transmission from cattle to humans of *E. coli* O157:H7 although these have not been confirmed by molecular typing [9–14]. Attempts to link and type *E. coli* O157:H7 strains from cattle and humans using various molecular methods may be hampered by the remarkable genotypic homogeneity shared among these strains. It is thought that strains of this serotype may be derived from a single highly conserved clone [15, 16]. Strains of *E. coli* O157:H7 from diverse sources have few characteristics that could be used to distinguish one strain from another reliably. Virulence factors include the production of one or more bacteriophage-encoded verocytotoxins, verotoxin 1 (VT1) and verotoxin 2 (VT2), and the ability to adhere intimately to the intestinal mucosa by an attaching and effacing mechanism which is partially mediated by the *eaeA* gene [2, 17, 18]. The presence of a high molecular weight plasmid in *E. coli* O157:H7 is highly associated with virulence, and is thought to mediate fimbrial adhesion [19], and encode a haemolysin [20].

Methods that have been used to type *E. coli* O157:H7 include verocytotoxin genotyping [21–23],

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plasmid profile analysis [22, 24], multilocus enzyme electrophoresis [16], phage typing [25–27], pulsed-field gel electrophoresis [5, 15, 27, 28], bacteriophage λ-restriction fragment length polymorphism profiling [29], ribotyping [30], and arbitrarily-primed PCR [31].

In this study, typing by PFGE was compared with plasmid profiling and phage typing on bovine isolates from different farms, and previously well characterized and epidemiologically unrelated *E. coli* O157:H7 isolates from both human and cattle sources. The application of PFGE to establish a link between cattle and human *E. coli* O157:H7 isolates from two separate farm outbreaks was evaluated. Determination of whether the presence of the *eaeA*, VT1 and VT2 genes, and the high MW 60 MDa virulence plasmid was more likely to be associated with human or bovine strains, was also made.

MATERIALS AND METHODS

Bacterial strains

A total of 51 *E. coli* O157:H7 strains were studied including 27 human isolates and 24 bovine isolates (Table 1). Seventeen isolates were from two separate farm outbreaks: Farm A, 10 isolates were collected from 2 cattle and 8 humans [32]; and Farm C, 7 isolates were collected from 2 humans and 2 bovine sources [13]. Study isolates were stored at −70 °C in buffered glycerol and subcultured twice on 5% sheep blood agar prior to use. The following strains were used as control strains: *E. coli* ATCC 25922 (VT1 and VT2 negative); and *E. coli* O157:H7 strain CL8 (VT1, VT2, and *eaeA* positive) [33].

Bacteriophage typing

Bacteriophage typing was performed using published methods, by the National Laboratory for Enteric Pathogens, Laboratory Centre for Disease Control in Ottawa, Ontario, Canada [25, 26].

Plasmid profile analysis and CVD419 determination

Plasmid DNA was extracted as described by Poppe and colleagues [34] and electrophoresed in a 0·7% agarose gel, stained with ethidium bromide and visualized by ultraviolet illumination. Molecular weight calculations were based on molecular weight standards included in each gel. Plasmid DNA was blotted onto nitrocellulose membranes [35]. To identify the plasmids having homology with the CVD419 probe (kindly provided by Dr J. P. Nataro), blots were hybridized with a 3·4 kb *Hin*dIII fragment, which was derived from the high MW plasmid present in strains of *E. coli* O157:H7 [19]. The probe was labelled with digoxigenin-11dUTP using the random primed method with the DIG DNA Labeling and Detection Kit (Boehringer–Mannheim, Laval, Quebec).

PFGE

Pulsed-field gel electrophoresis was performed according to the method of Bohm and Karch [15] with modifications. In brief, strains of *E. coli* O157:H7 were grown overnight in 5 ml of Luria–Bertani broth and centrifuged at 3000 ***g*** for 10 min. Pellets were resuspended in SE buffer (25 mM EDTA, 75 mM NaCl), and an equal volume of 1·6% low melt agarose was added. Plugs were set at 4 °C for 10 min and then lysed in lysis buffer (10 mM Tris–HCl, 50 mM NaCl, 100 mM EDTA, 0·2% deoxycholic acid, 1% sarkosyl, and 2 mg/ml lysozyme, Sigma) for 4 h at 37 °C with gentle agitation. After lysis, plugs were rinsed with TE buffer (10 mM Tris–HCl, and 0·1 mM EDTA) and then incubated at 42 °C overnight in proteinase K solution (100 mM EDTA, 0·2% deoxycholic acid, 1% sarkosyl, and 50 μg/ml proteinase K, Boehringer–Mannheim). After three washes with TE buffer, plugs were digested with *Xba*I (Boehringer–Mannheim) according to the manufacturer's instructions. DNA was then electrophoresed on a 1% agarose gel in a contour-clamped homogeneous electrical field using the CHEF-DR II (Bio-Rad, Mississauga, ON) with 0·5 × TBE buffer at 6 V/cm and 12 °C. Pulse times were linearly ramped from 3–35 s over 22 h. A bacteriophage lambda ladder (New England Biolabs, Mississauga, ON.) was used as a molecular size standard. Gels were then stained with ethidium bromide and photographed under UV illumination.

Strains with one or two band shifts consistent with a single genetic event were considered to be clonally related and subtypes of each other [36]. Strains that differed by three or more bands were considered to represent unique strains.

Restriction fragment length polymorphism (RFLP) analysis

After PFGE, DNA digested with *Xba*I underwent Southern hybridization using a 1·1 kb *eaeA* specific gene probe [33]. The probe was labelled with horseradish peroxidase using the ECL Direct Nucleic Acid

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Table 1. *Classification of 51* E. coli *O157:H7 isolates from humans and cattle by phage type, PFGE profile, plasmid profile and verotoxin genotype, Canada, 1986–94*

Phage type	PFGE *Xba*I profile* (no. isolates)	Plasmid profile (MDa)	Verotoxin profile†	No. of isolates‡	
				Human ($n = 27$)	Bovine ($n = 24$)
1	A(1)	70, 60, 4·5	1, 2	1	0
1	A(2)	60, 4·5	1, 2	1	1
1	A_1(1)	60, 4·5	1, 2	1	0
2	A(1)	60, 4·5	1, 2	1	0
2	B(1)	60	2	1	0
4	C(1)	60, 4·5	1, 2	1	0
4	C_1(1)	60	1, 2	1	0
8	D(6)	60, 4·5	1, 2	**4_{FA}**	**2_{FA}**
8	D_1(3), E(1)	60, 4·5	1, 2	**4_{FA}**	0
8	D_2(1), A_1(1)	60, 4·5	1, 2	2_{Fa}§	0
8	F(1)	60, 4·5	2	1	0
8	N.D.‖(1)	60, 4·5	1, 2	1	0
14	G(3)	70, 60	2	1	2_{FB}
14	H(1)	60	1	1	0
23	I(1)	60, 28, 4·5	1, 2	0	1_{FD}
23	I(2)	60, 28, 4·0	1, 2	0	2_{FD}
23	I(7)	60, 4·5	1, 2	**4_{FC}**	**2_{FC}**, 1
23	I_1(1)	60, 4·5	1, 2	**1_{FC}**	0
23	J(1)	60, 4·0	1, 2	0	1
32	K(3)	60, 2·1	1, 2	0	2, 1_{FD}
34	L(1)	60, 42, 4·5, 3·0	2	0	1
34	L(5)	60, 3·0	2	0	3_{FE}, 2
45	M(1)	60, 36, 4·5	1	1	0
67	I_1(1)	60, 45, 4·5, 1·7	1, 2	0	1
67	N(1)	60, 4·5, 2·6	1, 2	0	1
67	N(1)	60, 4·5	1, 2	0	1

* PFGE *Xba*I DNA profile: the lettered designate represents an unique DNA profile and the numerical subscript denotes a related subtype.

† Verotoxin genotype: 1 for VT1; 2 for VT2.

‡ Source of isolate is designated as farm 'FA' or 'FB' etc.; no designation is given for sporadic and epidemiologically unrelated isolates. Those isolates highlighted in bold type, belonged to the two separate outbreaks.

§ 'Fa' represents unrelated strains of the same phage type collected from the same surrounding region as isolates from farm 'FA'.

‖ N.D., not done.

Table 2. *Primers used in PCR for the amplification of VT1, VT2 and* eaeA *gene sequences for the* E. coli *O157:H7 isolate study, Canada, 1986–94*

Primers		Sequence 5′-3′	Location in gene	Reference
VT1	VT1c	ACCCTGTAACGAAGTTTGCG	31–50	[37]
	VT1d	ATCTCATGCGACTACTTGAC	151–170	
VT2	VT2a	TTAACCACACCCACGGCAGT	426–445	[38]
	VT2b	GCTCTGGATGCATGTCTGGT	752–771	
eaeA	C1	TCGTCACAGTTGCAGGCCTGGT	803–824	[33]
	C2	CGAAGTCTTATCCGCCGTAAAGT	1890–1912	

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labeling and detection system (Amersham, Oakville, ON) and detection of products was performed according to the manufacturer's instructions.

Polymerase chain reaction (PCR)

The presence of VT1, VT2 and *eaeA* genes was detected using primers as shown in Table 2. Template DNA was prepared by boiling several colonies of each isolate suspended in 50 μl of 1 × PCR buffer (Perkin–Elmer Cetus, Norwalk, CT) for 10 min. DNA was amplified in a 25 μl final reaction volume with 1 × PCR buffer, 200 μM of each dNTP, 2·5 U *Taq* DNA polymerase (Gibco–BRL, Burlington, ON), 5 pmol each of VT1 primers, 2·5 pmol of VT2 primers, 20 pmol of the *eaeA* gene primers (Gibco–BRL) and 1 μl of template DNA. Primers were designed to detect VT1 and VT2 gene sequences [37, 38], and the specific *eaeA* gene sequence of *E. coli* serotype O157:H7 [33]. Positive (*E. coli* O157:H7, strain CL8) and negative (*E. coli* ATCC 25922) controls were included with each PCR run, as well as reagent controls. Thermocycling conditions in a GeneAmp 9600 thermocycler (Perkin–Elmer Cetus) were as follows: 94 °C for 30 s, 56 °C for 30 s, and 72 °C for 30 s, for 30 cycles ending with a 3 min extension at 72 °C. PCR products were visualized on a 1·5% agarose gel after staining with ethidium bromide.

Discriminatory power of the typing methods

The discriminatory power of each typing method was determined by calculating the discriminatory index (DI) by the method of Hunter and Gatson [39].

RESULTS

Phage typing

Among the 51 *E. coli* O157:H7 isolates, 10 phage types were identified (no. of isolates in parentheses): phage types 1 (4), 2 (2), 4 (2), 8 (14), 14 (4), 23 (12), 32 (3), 34 (6), 45 (1) and 67 (3) (Table 1). The 10 outbreak isolates from Farm A belonged to phage type 8; and the 7 outbreak isolates from Farm C belonged to phage type 23.

Plasmid analysis

A total of 13 different plasmid profiles were observed and 55% (28/51) of the isolates had the plasmid profile, 60, 4·5 (Table 1). The distribution of plasmid profile patterns did not differ significantly between human and bovine strains. However, for phage type 8, all isolates had profile 60, 4·5. For isolates of phage 23, there were 4 plasmid profile patterns.

All human and bovine isolates tested, possessed a 60 MDa plasmid and were probe positive with the CVD419 DNA probe. Interestingly, only bovine isolates (50%) possessed plasmids of 4·0 MDa or less.

Strain differentiation by PFGE

PFGE with *Xba*I restriction endonuclease digestion generated 14 unique DNA profiles. Four PFGE profiles could be further divided into 9 subtypes. PFGE was much more discriminating than phage typing alone. For phage types 2, 8, 14, 23 and 67, PFGE generated at least two different DNA profiles within each phage type (Table 1). A single PFGE DNA profile was generated for the strains belonging to each of phage types 1, 4, 32, 34 and 45, respectively. PFGE profiles were not specific to any one phage type as strains of phage types 1 and 2 shared PFGE profile A; phage types 1 and 8 shared pattern A_1; and phage types 23 and 67 shared a similar pattern, I_1. More than one PFGE pattern could be identified for isolates with a single plasmid profile, and more than one plasmid profile could be identified for isolates of a single PFGE pattern.

The DI for phage typing, PFGE, plasmid profiling and verotoxin genotyping were calculated to be 0·85, 0·93, 0·69 and 0·40, respectively.

Molecular typing of outbreak strains

The outbreak strains from each farm had identical PFGE patterns (Fig. 1). For the outbreak on Farm A due to phage type 8, 2 bovine isolates and 4 human isolates had an identical PFGE pattern D, which correlated with phage typing, plasmid profiling and verotoxin genotyping results. Additionally, 4 other human isolates associated with this farm outbreak, had PFGE patterns D_1 (3 isolates) and E (1 isolate). Two other human phage type 8 isolates, unrelated to the outbreak but originating from the same geographic region, had PFGE patterns D_2 and A_1.

For the Farm C outbreak, the *E. coli* O157:H7 isolate belonging to the index case had PFGE pattern I_1. Four isolates from one other human (sibling with symptomatic diarrhoea) and two bovine isolates of phage type 23, were typed as having PFGE pattern I. Plasmid profiling and verotoxin genotyping were

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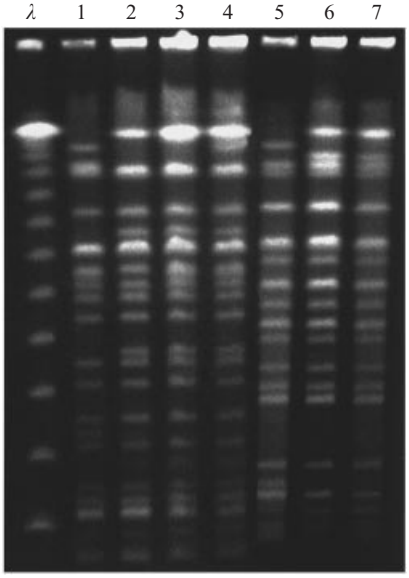

Fig. 1. Agarose gel showing PFGE profiles of *Xba*I-digested DNA of outbreak isolates from Farms A (Ontario, Canada, 1986) and C (Ontario, Canada, 1992). Farm A: lane 1, human isolate with profile E; lane 2, human isolate with profile D; lanes 3, 4, bovine isolates with profile D. Farm C: lane 5, human isolate with profile I_1; lanes 6, 7, human and bovine isolate with profile I. λ, lambda ladder (New England Biolabs).

identical for these outbreak phage type 23 isolates. Four unrelated bovine phage type 23 isolates, differed from the outbreak strains, either by plasmid profiles or PFGE profiles.

Toxin genotypes and *eaeA* determination

Of the 27 human isolates tested, 22 (81·5%) were VT1 and VT2 positive; 2 (7·5%) were VT1 positive; and 3 (11%) were VT2 positive. Of the 24 bovine isolates, 16 (67%) were positive for both VT1 and VT2; and 8 (33%) were positive only for VT2. The proportion of isolates containing both toxin gene sequences was not significantly different between strains of human and bovine origin (Fisher's exact test). The *E. coli* O157:H7 *eaeA* specific gene sequence was detected in all study isolates.

Restriction fragment length polymorphism using a 1·1 kb *eaeA* gene probe of Southern blotted PFGE *Xba*I restricted DNA was not very helpful for strain differentiation. All *E. coli* O157:H7 isolates showed the same probe pattern except for one phage type 2 isolate with PFGE pattern B, which had a different RFLP pattern (data not shown).

DISCUSSION

Until recently, the epidemiological investigations of *E. coli* O157:H7 infections have relied on detection of VT production, culture isolation, and phage typing [9, 12, 14, 32]. Molecular typing has improved our knowledge of the epidemiology of *E. coli* O157:H7 infection and their mechanisms of transmission [5, 15, 27, 40, 41]. *E. coli* O157:H7 is known to be a food-borne pathogen and the reservoir is thought to be in cattle [1, 2, 9–11]. Although molecular typing studies have shown the direct transmission of *E. coli* O157:H7 from contaminated foods to humans [40, 41], and person to person transmission [7], few studies have shown the direct transmission of *E. coli* O157:H7 from cattle to humans [13, 32].

PFGE, phage typing, plasmid profile analysis and VT gene profiles were used to study the molecular epidemiology of *E. coli* O157:H7 transmission from cattle to humans. In this study, isolates were collected from two previously documented *E. coli* O157:H7 outbreaks, and from a collection of human and cattle isolates of *E. coli* O157:H7 belonging to different phage types. Plasmid profiling alone did not provide sufficient discriminatory power since more than 50% of isolates of diverse phage and PFGE types, harboured two plasmids of 60 and 4·5 MDa. RFLP using the *eaeA* gene probe was of limited value since all except one study isolate demonstrated an identical profile; strains may have acquired the *eaeA* gene in the same genetic locus during the same evolutionary process.

PFGE was capable of differentiating between epidemiologically related and unrelated strains of *E. coli* O157:H7 (diversity index = 0·93). In many instances, PFGE was able to further differentiate related strains into subtypes; and PFGE was used as a tool to verify the direct transmission of *E. coli* O157:H7 from cattle to humans. Phage types 8 and 23 were responsible for the outbreaks on Farm A [32] and Farm C [13], respectively. *E. coli* O157:H7 isolates belonging to 4 of the 8 individuals associated with the outbreak on Farm A, shared identical PFGE profiles with isolates from cattle from the same farm. For both outbreaks, each farm clearly had cattle strains that shared identical PFGE profiles with the human strains related to the outbreaks, implicating direct cattle-to-human transmission. This report lends further support and provides the molecular evidence for the direct cattle-to-human transmission of *E. coli* O157:H7. PFGE has shown that more than one *E.*

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coli O157:H7 strain with varying DNA subtypes, may be responsible for an outbreak, and that more than one *E. coli* O157:H7 subtype may be circulating on a particular farm at any one time. Human disease may be associated with different subtypes of the same PFGE DNA profile and PFGE subtypes of the same *E. coli* O157:H7 strain could be in existence on a farm with endemic *E. coli* O157:H7. This is supported by the findings of Karch and colleagues who documented clonal turnover, that is the occurrence of a change in PFGE patterns, within a host over time [42]. Although the outbreak strains were not identical by PFGE, the strains of phage types 23 and 8, were related as evidenced by only a two band difference between PFGE profiles I and I_1, and between PFGE profiles D and D_1, respectively. Moreover, Tenover's criteria specifying strain identity still considers an isolate to be possibly related to the outbreak strain even if its PFGE pattern differs form the outbreak pattern by 4–6 bands [43]. However, Barrett and colleagues have suggested that isolates with PFGE DNA profiles that differed from the PFGE pattern of the outbreak strain by just one band were probably not related [28]. They thought that due to the clonal nature of *E. coli* O157:H7 strains, the PFGE patterns should be very highly conserved. They conceded that although PFGE patterns of outbreak strains, differing by a single band may not be sufficient to suggest relatedness, such classification should not be based on PFGE alone. Barrett and colleagues suggested that phage typing would provide additional evidence to support whether outbreak strains are related or not [28].

Paros and colleagues found that 90% of human *E. coli* O157:H7 isolates did not match any of the bovine strains studied [29]. However, they examined epidemiologically unrelated human and bovine strains. They cite insensitive methods of strain differentiation, poor reproducibility of typing methods, and the genetic alteration of human *E. coli* O157:H7 isolates after leaving the bovine reservoir, as possible reasons for the discrepancy between human and bovine typing results.

However, in the study presented here, epidemiologically related human and bovine strains were investigated. As well, PFGE DNA profiles were found to be consistently stable and reproducible on separate occasions and even after repeated subculturing (data not shown). This study was limited by having only a few human and bovine strains that shared the same PFGE subtype pattern in each outbreak.

In summary, the findings in this study provide further evidence to support the direct transmission from cattle to humans of *E. coli* O157:H7 infection. At any one time, more than one *E. coli* O157:H7 PFGE type and subtype may prevail in an outbreak and careful typing strategies are needed to identify the outbreak strain. Recent work by Michel and colleagues has demonstrated that sporadic cases of human *E. coli* O157:H7 infection are linked to areas with high cattle density [44]. Techniques such as PFGE will be required to further investigate routes of transmission and development of appropriate interventions to reduce sporadic *E. coli* O157:H7 infection. PFGE is a useful molecular typing method for differentiating among *E. coli* O157:H7 strains but additional typing methods may be occasionally needed in outbreak investigations, given the genetic homogeneity of these bacteria and the possible occurrence of clonal turnover [28, 42].

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REFERENCES

1. Griffin PM, Tauxe RV. The epidemiology of infections caused by *Escherichia coli* O157:H7, other enterohemorrhagic *E. coli*, and the associated hemolytic uremic syndrome. Epidemiol Rev 1991; **13**: 60–98.
2. Karmali MA. Infection by verocytotoxin-producing *Escherichia coli*. Clin Microbiol Rev 1989; **2**: 15–38.
3. Anonymous. Outbreak of *Escherichia coli* O157:H7 infections associated with drinking unpasteurized commercial apple juice – British Columbia, California, Colorado, and Washington. MMWR 1996; **45**: 975.
4. Bell BP, Goldoft M, Griffin PM, et al. A multistate outbreak of *Escherichia coli* O157:H7 – associated bloody diarrhea and hemolytic uremic syndrome from hamburgers: the Washington experience. JAMA 1994; **272**: 1349–53.
5. Izumiya H, Terajima J, Wada A, et al. Molecular typing of enterohemorrhagic *Escherichia coli* O157:H7 isolates in Japan by using pulsed-field gel electrophoresis. J Clin Microbiol 1997; **35**: 1675–80.
6. Anonymous. Enterohemorrhagic *Escherichia coli* in-

fection – Japan. WHO Weekly Epidemiol Rec 1996; **71**, no. 35.
7. Ludwig K, Ruder H, Bitzan M, Zimmermann S, Karch H. Outbreak of *Escherichia coli* O157:H7 infection in a large family. Eur J Clin Microbiol Infect Dis 1997; **16**: 238–41.
8. Belongia EA, Osterholm MT, Soler JT, Ammend DA, Braun JE, MacDonald KL. Transmission of *Escherichia coli* O157:H7 infection in Minnesota child day-care facilities. JAMA 1993; **269**: 883–8.
9. Wells JG, Shipman LD, Greene KD, et al. Isolation of *Escherichia coli* serotype O157:H7 and other Shiga-like-toxin-producing *E. coli* from dairy cattle. J Clin Microbiol 1991; **29**: 985–9.
10. Borczyk AA, Karmali MA, Lior H, Duncan LMC. Bovine reservoir for verotoxin-producing *Escherichia coli* O157:H7. Lancet 1987; i: 98.
11. Heuvelink AE, Van Den Biggelaar FLAM, De Boer E, et al. Isolation and characterization of verocytotoxin-producing *Escherichia coli* O157 strains from Dutch cattle and sheep. J Clin Microbiol 1998; **36**: 878–82.
12. Besser TE, Hancock DD, Pritchett LC, McRae EM, Rice DH, Tarr PI. Duration of detection of fecal excretion of *Escherichia coli* O157:H7 in cattle. J Infect Dis 1997; **175**: 726–9.
13. Renwick SA, Wilson JB, Clarke RC, et al. Evidence of direct transmission of *Escherichia coli* O157:H7 infection between calves and a human. J Infect Dis 1993; **168**: 792–3.
14. Martin ML, Shipman LD, Wells JG, et al. Isolation of *E. coli* O157:H7 from dairy cattle associated with two cases of haemolytic uremic syndrome. Lancet 1986; ii: 1043.
15. Bohm H, Karch H. DNA fingerprinting of *Escherichia coli* O157:H7 strains by pulsed-field gel electrophoresis. J Clin Microbiol 1992; **30**: 2169–72.
16. Whittam TS, Wachsmuth IK, Wilson RA. Genetic evidence of clonal descent of *Escherichia coli* O157:H7 associated with hemorrhagic colitis and hemolytic uremic syndrome. J Infect Dis 1988; **157**: 1124–33.
17. O'Brien AD, Holmes RK. Shiga and Shiga-like toxins. Microbiol Rev 1987; **51**: 206–20.
18. McKee ML, O'Brien AD. Truncated enterohemorrhagic *Escherichia coli* (EHEC) O157:H7 intimin (*eaeA*) fusion proteins promote adherence of EHEC strains to HEp-2 cells. Infect Immun 1996; **64**: 2225–33.
19. Levine MM, Xu J, Kaper JB, et al. A DNA probe to identify enterohemorrhagic *Escherichia coli* of O157:H7 and other serotypes that cause hemorrhagic colitis and hemolytic uremic syndrome. J Infect Dis 1987; **156**: 175–82.
20. Schmidt H, Karch H, Beutin L. The large-sized plasmids of enterohemorrhagic *Escherichia coli* O157 strains encode hemolysins which are presumably members of the *E. coli* α-hemolysin family. FEMS Microbiol Lett 1994; **117**: 189–96.
21. Samadpour M. Molecular epidemiology of *Escherichia coli* O157:H7 by restriction fragment length polymorphism using Shiga-like toxin genes. J Clin Microbiol 1995; **33**: 2150–4.
22. Ostroff SM, Tarr PI, Neill MA, Lewis JH, Hargrett-Bean N, Kobayashi JM. Toxin genotypes and plasmid profiles as determinants of systemic sequelae in *Escherichia coli* O157:H7 infections. J Infect Dis 1989; **160**: 994–8.
23. Newland JW, Neill RJ. DNA probes for Shiga-like toxins I and II and for toxin-converting bacteriophages. J Clin Microbiol 1988; **26**: 1292–7.
24. Wells JG, Davis BR, Wachsmuth IK, et al. Laboratory investigation of hemorrhagic colitis outbreaks associated with a rare *Escherichia coli* serotype. J Clin Microbiol 1983; **18**: 512–20.
25. Khakhria R, Duck D, Lior H. Extended phage-typing scheme for *Escherichia coli* O157:H7. Epidemiol Infect 1990; **105**: 511–20.
26. Ahmed R, Bopp C, Borczyk A, Kasatiya S. Phage-typing scheme for *Escherichia coli* serotype O157:H7. J Infect Dis 1987; **155**: 806–9.
27. Krause U, Thomson-Carter FM, Pennington TH. Molecular epidemiology of *Escherichia coli* O157:H7 by pulsed-field gel electrophoresis and comparison with that by bacteriophage typing. J Clin Microbiol 1996; **34**: 959–61.
28. Barrett TJ, Lior H, Green JH, et al. Laboratory investigation of a multistate food-borne outbreak of *Escherichia coli* O157:H7 by using pulsed-field gel electrophoresis and phage typing. J Clin Microbiol 1994; **32**: 3013–7.
29. Paros M, Tarr PI, Kim H, Besser TE, Hancock DD. A comparison of human and bovine *Escherichia coli* O157:H7 isolates by toxin genotype, plasmid profile, and bacteriophage λ-restriction fragment length polymorphism profile. J Infect Dis 1993; **168**: 1300–3.
30. Martin IE, Tyler SD, Tyler KD, Khakhria R, Johnson WM. Evaluation of ribotyping as epidemiologic tool for typing *Escherichia coli* serogroup O157 isolates. J Clin Microbiol 1996; **34**: 720–3.
31. Madico G, Akopyants NS, Berg DE. Arbitrarily primed PCR DNA fingerprinting of *Escherichia coli* O157:H7 strains by using templates from boiled cultures. J Clin Microbiol 1995; **33**: 1534–6.
32. Duncan L, Mai V, Carter A, Carlson JAK, Borczyk A, Karmali MA. Outbreak of gastrointestinal disease – Ontario. Can Dis Wkly Rep 1987; **13-2**: 5–8.
33. Louie M, De Azavedo J, Clarke R, et al. Sequence heterogeneity of the *eae* gene and detection of verotoxin-producing *Escherichia coli* using serotype-specific primers. Epidemiol Infect 1994; **112**: 449–61.
34. Poppe C, Irwin RJ, Forsberg CM, Clarke RC, Oggel J. The prevalence of *Salmonella enteritidis* and other *Salmonella* spp. among Canadian registered commercial layer flocks. Epidemiol Infect 1991; **106**: 259–70.
35. Sambrook J, Fritsch EF, Maniatis T. Molecular cloning. In: Laboratory manual, 2nd edn, Cold Spring Harbor: Cold Spring Harbor Laboratory Press, N.Y., 1989: 9.38–9.40.
36. Maslow JN, Slutsky AM, Arbeit RD. Application of pulsed-field gel electrophoresis for molecular epidemiology. In: Persing DH, Smith TF, Tenover FC, White

TJ, eds. Diagnostic molecular microbiology principles and applications. Washington, D.C.: American Society for Microbiology, 1993: 563–72.
37. Pollard DR, Johnson WM, Lior H, Tyler SD, Rozee KR. Differentiation of Shiga toxin and verocytotoxin type 1 genes by polymerase chain reaction. J Infect Dis 1990; **162**: 1195–8.
38. Pollard DR, Johnson WM, Lior H, Tyler SD, Rozee KR. Rapid and specific detection of verotoxin genes in *Escherichia coli* by the polymerase chain reaction. J Clin Microbiol 1990; **28**: 540–5.
39. Hunter PR, Gaston MA. Numerical index of the discriminatory ability of typing systems: an application of Simpson's index of diversity. J Clin Microbiol 1988; **26**: 2465–6.
40. Grimm LM, Goldoft M, Kobayashi J, et al. Molecular epidemiology of fast-food restaurant-associated outbreak of *Escherichia coli* O157:H7 in Washington State. J Clin Microbiol 1995; **33**: 2155–8.
41. Bender JB, Hedberg CW, Besser JM, Boxrud DJ, MacDonald KL, Osterholm MT. Surveillance for *Escherichia coli* O157:H7 infections in Minnesota by molecular subtyping. N Engl J Med 1997; **337**: 388–94.
42. Karch H, Russmann H, Schmidt H, Schwarzkopf A, Heesemann J. Long-term shedding and clonal turnover of enterohemorrhagic *Escherichia coli* O157 in diarrheal diseases. J Clin Microbiol 1995; **33**: 1602–5.
43. Tenover FC, Arbeit RD, Goering RV, et al. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J Clin Microbiol 1995; **33**: 2233–9.
44. Michel P, Wilson J, Martin W, McEwen S, Gyles C, Clarke R. Temporal and spatial distributions of human cases of verocytotoxigenic *Escherichia coli* infection in southern Ontario. In: Abstracts and Proceedings of the Third International Symposium on *Shiga*-like producing *Escherichia coli*, Baltimore, Maryland. 22–26 June 1997; Abstract no: V90/I: 12.