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ORIGINAL ARTICLE

Soil survival of *Escherichia coli* O157:H7 acquired by a child from garden soil recently fertilized with cattle manure

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Abstract

Aims: This investigation was conducted to determine the survival of a naturally occurring *Escherichia coli* O157:H7 in garden soil linked to a sporadic case of *E. coli* O157 infection in Minnesota.

Methods and Results: The presence and viability of *E. coli* O157:H7 was monitored in manure-contaminated garden soil for several weeks. Bacterial isolates were characterized using PCR and pulsed-field gel electrophoresis (PFGE). Isolates obtained from the patient and the garden plots during this investigation had indistinguishable PFGE patterns and had the same virulence factors (*stx*1, *stx*2, *eae*A, *ehx*A). The *E. coli* O157:H7 levels obtained from the garden plots declined gradually for a period of 2 months, and on day 69 only one garden plot of four had detectable levels of pathogen. All plots were negative on day 92. The rate of decline in the soil samples stored at 4°C was faster compared with soil samples that remained in ambient conditions, and in refrigerated storage *E. coli* O157:H7 could not be detected after 10 days.

Conclusions: *E. coli* O157:H7 strains can survive on manure-amended soil for more than 2 months, and this survival could be reduced by low temperature.

Significance and Impact of the Study: This is one of the few reports that have investigated the survival of a proven virulent strain in naturally contaminated soil samples. This case stresses the importance of avoiding the use of raw cattle manure to amend soil for cultivation of foods, including soils in residential garden plots.

Introduction

Escherichia coli O157:H7 is a pathogenic bacterium that causes enterohaemorrhagic infections. According to the Centers for Disease Control and Prevention, this organism is responsible for more than 73 000 human cases every year in the United States, and the majority of those infections are sporadic cases (Mead *et al.* 1999). In recent years, however, there has been a decline in the incidence of *E. coli* O157:H7 (CDC 2005). Infections caused by *E. coli* O157:H7 are typically associated with the consumption of contaminated foods, but approximately half of all outbreaks are transmitted by other routes such as water and person to person (Rangel *et al.* 2005). Because cattle are a primary natural reservoir, the risk factors identified for sporadic cases include consumption of poorly cooked ground beef, visiting and living on a farm, living in agricultural areas, contact with farm animals and contact with cattle manure (Michel *et al.* 1999; Kassenborg *et al.* 2004).

Cattle and sheep manure can serve as the vehicle for *E. coli* O157:H7 contamination of fruits, vegetables, water and soil (Hilborn *et al.* 1999; Chalmers *et al.* 2000; Rangel *et al.* 2005). This pathogen can also be transmitted directly from manure; this infection route has been implicated in several outbreaks (Duffy 2003). In 2000, 20

individuals in Scotland contracted enterohaemorrhagic colitis after camping on a field that had been recently grazed by sheep (Strachan *et al.* 2001; Howie *et al.* 2003). The outbreak investigation determined that the infection route was directly from manure-containing soil. In Austria, two children developed bloody diarrhoea from farm fields contaminated by cattle manure after they were exposed to the environment of an adjacent farm (Grif *et al.* 2005). Detailed reports of cases of direct transmission of *E. coli* O157:H7 from manure are, however, not very common in the literature.

Experiments that used *E. coli* O157:H7 cultures have indicated that this organism can remain viable in manure and soil for long periods of time (Wang *et al.* 1996; Maule 2000). Using inoculated samples, a maximum survival time of 70 days in manure was observed by Wang *et al.* (1996) and 130 days in soil was first reported by Maule (2000). Some reports have indicated that the survival of *E. coli* O157:H7 in manure was enhanced by low temperature (Wang *et al.* 1996; Himathongkham *et al.* 1999); however, a recent report observed a reduced survival at 5°C when compared with 15 or 21°C (Jiang *et al.* 2002). Additional factors that influence pathogen survival in the environment include type of soil and source of manure (Hutchison *et al.* 2004; Islam *et al.* 2004). Most reports that have investigated the survival of *E. coli* O157:H7 in the environment have used samples that were inoculated with pure cultures; few studies have monitored the survival of naturally present pathogenic strains.

This investigation was undertaken to determine the survival of a naturally occurring *E. coli* O157:H7 strain in manure-amended soil that was the source of a sporadic case of *E. coli* O157:H7 infection in a child in Minnesota.

Materials and methods

Case ascertainment and background

On 25 April 2002, a physician reported an *E. coli* O157 : H7 infection in a 2-year-old patient to the Minnesota Department of Health (MDH). The patient's parents reported to MDH that 4 days before onset of illness in the case patient, they fertilized the soil in the garden plots at their suburban home with raw cow manure obtained from a relative's farm in Wisconsin. One day after amending the soil with the manure, the family planted radishes; the case patient played on the plots while the parents did the planting. The MDH Public Health Laboratory (MDH PHL) isolated *E. coli* O157:H7 from the soil samples collected from three of the four garden plots 1 week after the case patient's illness onset. All soil isolates were indistinguishable from the case patient's isolate by pulsed-field gel electrophoresis (PFGE) conducted at the MDH PHL; the PFGE subtype was given the designation MN628 and submitted to the PulseNet database (Fig. 1). Based on the comparison with PFGE subtypes in the PulseNet database, this subtype had never been previously found among isolates from human, animal or environmental sources in the United States. On the basis of this information, the University of Minnesota, Department of Food Science and Nutrition was consulted to evaluate the ongoing status of the garden plots with respect to contamination with *E. coli* O157:H7.

Collection of soil and radish samples

The residential garden had three plots in its backyard (plots 1, 2 and 3) approx. 4 × 2 m in size and one larger plot (plot 4) approx. 5 × 6 m in size just adjacent to the house. All four plots were amended with raw manure by the homeowners on 21 April 2002. A composite sample of 1 kg from each plot was collected during each visit (days 19, 42, 69, 92 and 356 after the soil was amended with manure) by taking random portions of soil at different locations and depths with a sterile scoop. The garden plots were not treated or cultivated during the study period. Radishes were collected at day 69 when they were ready for harvest. One composite sample of radishes was taken from each plot. Approximately, ten individual plants from each plot were picked randomly and placed

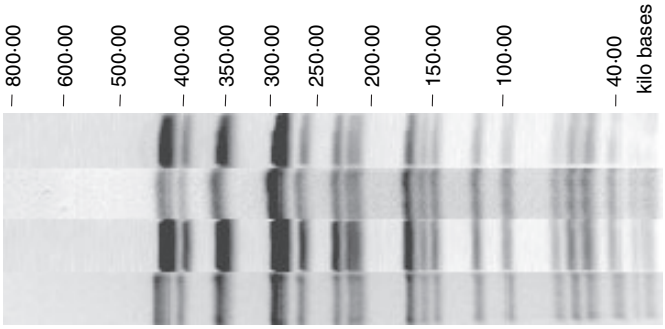

Figure 1 Pulsed-field gel electrophoresis analysis of representative *Escherichia coli* O157:H7 isolates obtained from the garden plots and the patient.

into sterile bags. Samples were immediately transported to the Food Safety Microbiology Laboratory at the University of Minnesota and analysed the same day. Radish samples were not brushed or rinsed before analyses and all the portions of the radish plants were tested.

Escherichia coli O157:H7 and coliform analysis

Analyses of coliforms were conducted as follows: 25 g of samples were mixed with 225 ml of lauryl sulfate tryptose (LST) broth (Neogen Inc., Lansing, MI, USA) in a stomacher (Tekmar Co., Cincinnati, OH, USA) for 2 min. Coliform counts were determined by the three-tube most-probable-number (MPN) technique using three tenfold serial dilutions in LST broth incubated at 37°C for 48 h. A loop full of enrichment from LST tubes, showing growth and gas production, was transferred to 9-ml brilliant green bile broth (BGBB; Neogen Inc.) containing lactose for selective enrichment of coliforms (Feng *et al.* 2002). BGBB tubes showing growth and gas production were used for the determination of MPN for coliform contamination in the soil samples.

The qualitative analysis of *E. coli* O157:H7 was a modified method of that described previously (Omisakin *et al.* 2003) and started by blending 25 g of soil (either recently collected or stored at 4°C) or radish samples into 225 ml of tryptic soy broth (TSB; Neogen Inc.) supplemented with 20 mg l^{-1} novobiocin (ICN Biomedicals Inc., Irvine, CA, USA), which was incubated at 42°C for 6 h. These TSB cultures (0·5 ml) were mixed with 10 μl of Dynal bead suspensions coated with anti-O157 antibodies (Dynal Inc., Lake Success, NY, USA) and incubated for 30 min with gentle motion at room temperature. Tubes containing the culture/beads mixture were then placed in a strong magnet (Miltenyi Biotec Inc., Auburn, CA, USA) for 5 min, and the supernatant liquid was discarded. The beads were then suspended in 0·5 ml of buffered peptone water (BPW) containing 0·05% Tween 20, incubated for 5 min with gentle motion, placed in the magnet for 5 min and the liquid removed. These washing steps were repeated two more times, after which the beads were re-suspended in 100 μl of BPW and plated on CHROMagar O157 (CHROMagar Microbiology, Paris, France) supplemented with 2·5 mg l^{-1} potassium tellurite (ICN Biomedicals Inc.). Depending on the number of mauve/purple-coloured colonies, one to ten of those colonies per plate were tested for O157 antigen using an *E. coli* O157 latex agglutination kit (Oxoid Ltd, Hampshire, UK). *Escherichia coli* O157:H7 strain ATCC 43895 was used as a positive control; it was confirmed that this method could detect as few as 10 cells per 25 g of sample (data not shown).

Enumeration of *E. coli* O157:H7 in recently collected or stored soil samples at 4°C was carried out by plating and measuring counts of the pathogenic strain in serial dilutions of the soil samples (Feng and Weagant 2002). Twenty-five grams of samples were blended well in 225 ml of brain heart infusion (BHI) broth (Neogen, Inc.) as described above. BHI homogenates were serially diluted from 10^{-2} to 10^{-5} in 9-ml BHI tubes, and 0·1 ml of each dilution was plated, in duplicate, on CHROMagar O157 supplemented with 2·5 mg l^{-1} potassium tellurite. Depending on the number of colonies, one to ten mauve/purple-coloured colonies per plate were confirmed by latex agglutination test as described above. The numbers of *E. coli* O157:H7 were determined by counting the mauve/purple colonies using a manual colony counter (Gallenkamp, England) and averaged.

Identification of phenotypic traits and virulence genes

For each O157-agglutination-positive isolate, the H7 antigen was determined using a RIM®-O157 latex agglutination kit (Remel Inc., Lenexa, KS, USA). Isolates were struck onto sorbitol McConkey agar plates and incubated for 24 h at 37°C to determine their ability to use sorbitol. The presence of virulence genes was determined using PCR with a Robocycler® thermal system (Stratagene Inc., La Jolla, CA, USA) to detect virulence marker genes using specific primers for Shiga-like toxins (*stx*1 and *stx*2), intimin (*eaeA*), and enterohaemolysin (*ehx*A). Bacterial cells used to derive the DNA template were grown in LB overnight at 37°C. One millilitre of the broth was pipetted into a microcentrifuge tube and placed in a boiling water bath for 20 min. The microcentrifuge tubes were then put on ice for 5 min and finally centrifuged at 20 000 ***g*** for 2 min, and the supernatant was used as the source of DNA template. The PCR primers and conditions are described in Table 1.

Chromosome-encoded virulence genes (*stx*1, *stx*2 and *eaeA*) and plasmid-encoded virulence genes (*ehx*A) were detected using PCR amplification. A total 50-μl volume of PCR reaction mixture containing a 2·0 mmol l^{-1} concentration of deoxynucleoside triphosphate, a 1 μmol l^{-1} concentration of each primer, 5 μl of 10× PCR buffer and 1·25 U of *Taq* DNA polymerase (Promega, Madison, WI, USA) with the addition of 2 μl DNA extracts from the supernatant of bacterial cell suspension after centrifugation were used in the experiment. The PCR reaction products of the samples were then electrophoresed on a 0·8% agarose (Sigma Inc., St. Louis, MO, USA) gel. The gels were stained with a 0·5 mg l^{-1} ethidium bromide (ICN Biomedicals Inc.) solution, destained with distilled water and a picture was taken with a Gel-Doc 8000 Gel Documentation System (UVP Inc., Upland, CA, USA). Lambda DNA ladder from 0·2 to 2 kb (Gibco/Life Technologies, Carlsbad, CA, USA) was used as molecular weight standards.

Table 1 PCR primers and conditions used

Primer sequences (5′–3′)	Gene	PCR conditions			Product size (bp)	References
		Denaturation time at 94°C (s)	Annealing	Extension time at 72°C (s)		
CAGTTAATGTGGTGGCGAAGG CACCAGACAATGTAACCGCTG	*stx*I	60	60°C, 60 s	60	348	Cebula *et al.* (1995)
ATCCTATTCCCGGGAGTTTACG GCGTCATCGTATACACAGGAGC	*stx*II	60	60°C, 60 s	60	584	Cebula *et al.* (1995)
CAGGTCGTCGTGTCTGCTAAA TCAGCGTGGTTGGATCAACCT	*eae*A	60	60°C, 60 s	60	1087	Gannon *et al.* (1993)
GGTGCAGCAGAAAAAGTTGTAG TCTCGCCTGATAGTGTTTGGTA	*ehx*A	40	55°C, 90 s	100	1551	Schmidt *et al.* (1995)

Final extension was carried out at 72°C for 10 min.

Pulsed-field gel electrophoresis

The standardized PulseNet's laboratory protocol was used with minor modifications as previously described (Bender *et al.* 1997). Bacterial strains were grown overnight on TSA plates at 37°C. Bacterial colonies were suspended in cell suspension buffer (100 mmol l^{-1} Tris, 100 mmol l^{-1} EDTA, pH 8·0) and adjusted to an optical density of 1·3–1·4 using a spectrophotometer (Bio-Rad Laboratories, Hercules, CA, USA). The 400-μl adjusted cell suspension was mixed with 20 μl of proteinase K (20 mg ml^{-1} stock) and an equal volume (400 μl) of melted 1% SeaKem Gold Agarose (BioWhittaker, Rockland, ME, USA) containing 1% sodium dodecyl sulfate. The mixture was carefully dispensed into appropriate wells of a reusable plug mould (Bio-Rad Laboratories). After solidification, the plugs were transferred to each of the round bottom tubes containing 1·5 ml of cell lysis buffer (50 mmol l^{-1} Tris–HCl, 50 mmol l^{-1} EDTA, pH 8·0; 1% sarcosine) and 0·5 mg ml^{-1} of proteinase K. Cells were lysed in a 54°C water bath for 2 h with constant and vigorous agitation at 175–200 rev min^{-1}. After lysis, the plugs were washed twice with preheated water and four times with preheated TE buffer (10 mmol l^{-1} Tris, 1 mmol l^{-1} EDTA, pH 8·0) for 10–15 min per wash at 50°C with the same agitation. Plugs were stored in 2 ml of TE buffer at 4°C until it was ready for restriction digestion of DNA. DNA in agarose plugs was digested with 50 U $(sample)^{-1}$ of *Xba*I (Promega) for at least 2 h in a 37°C water bath. The plugs were loaded onto the wells in a 1% pulse-field-certified agarose gel. DNA restriction fragments were separated with a CHEF-DR® III (Bio-Rad Laboratories) with pulse times of 5–50 s at 14°C for 18 h in 0·5× TBE buffer at 6 V cm^{-1}. The gel was stained with ethidium bromide (Sigma-Aldrich), and restriction fragment patterns were photographed with a UV transilluminator. *Escherichia coli* G5244 strain was used as a standard control in this analysis.

Pulsed-field gel electrophoresis band patterns were compared using guidelines for interpreting PFGE-generated DNA patterns, as described by Tenover *et al.* (1995). According to Tenover *et al.*, an isolate is considered indistinguishable from the outbreak strain if it has the same number and size of bands in a PFGE pattern, and it is designated as Type A. The same report also defines that 'an isolate is considered to be closely related to the outbreak strain if its PFGE pattern differs from the outbreak pattern by changes consistent with a single genetic event'. Those strains are designated 'Type A1' and typically do not have more than three bands different than the outbreak strain.

Statistical analysis

All analyses (PFGE, culture isolation and PCR) were performed in duplicate, and the data shown in the graphs are the average of two separate measurements conducted in duplicate. Decimal reduction times (D) were calculated using regression coefficient analysis. SAS version 9.1 (SAS Institute Inc., Cary, NC, USA) was used to determine the statistical differences between the counts using Student's t-test for calculating the regression coefficients and for determining the R values.

Results

Nineteen days after the soil was amended with manure, samples from all four plots had an average level of approx. 3·4 log CFU g^{-1} of *E. coli* O157:H7 and a coliform count of 5 log MPN g^{-1} (Table 2). The coliform counts in soil samples collected over the following 13 weeks declined substantially, but at least one sample still had approx. 1·5 log CFU g^{-1} after 92 days (Table 2). Over the same period, the number of positive samples and counts of *E. coli* O157:H7 declined. This pathogen

Table 2 Coliform and *Escherichia coli* O157:H7 populations in manure-amended garden soil samples

	Time after manure application to soil (days)				
Bacteria	19	42	69	92	356
Coliforms (log MPN g^{-1})	5·0[a]	3·4[b]	2·3[b]	0·9[c]	0·1[c]
E. coli O157:H7 (log CFU g^{-1})*	3·4[a] (4)	1·9[b] (2)	1·0[bc] (1)	0·0[c] (0)	0·0[c] (0)

Data are the mean of coliform and *E. coli* O157:H7 counts for sample plots that tested positive. Means not sharing the same letters were significantly different ($P < 0·01$).

*Number in parentheses is the number of plots that had positive samples before they were quantified.

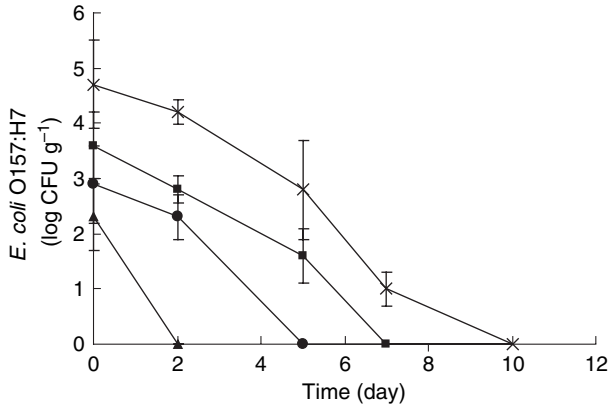

Figure 2 Survival of *Escherichia coli* O157:H7 in soil samples collected 19 days after manure application and stored at 4°C. ●, Plot1; ■, plot2; ▲, plot3; ×, plot4.

was present in two of the four garden plots on day 42, one of the plots on day 69 and none of the plots on day 92. None of the soil samples from the same four locations tested positive for the pathogen after approx. 1 year without any treatment and cultivation. At day 69, the first day we observed that most of the radishes were ripe and ready to be harvested, analysis were conducted and none of the four composite samples tested positive for *E. coli* O157:H7.

Soil samples used for survival studies were conducted at 4°C to determine whether low temperature could prolong the viability of *E. coli* O157:H7, when compared with ambient conditions. The survival of *E. coli* O157:H7 present in the soil samples collected at 19 days after amending manure into the garden soil and stored at 4°C was dependent upon the initial count (Fig. 2). *Escherichia coli* O157:H7 counts declined rapidly at approximately the same rate of reduction within 10 days of storage in all of the soil samples collected from the garden plots (Fig. 2). The decimal reduction time (D) determined in the samples taken from plots 1, 2 and 4 were 40·3, 48·3 and 47·7 h, respectively ($R^2 \geq 0·96$). The pathogen survived longest in the soil sample that had an average of 4·7 log CFU g^{-1} collected from plot 4 but could not be detected after 10 days of refrigerated storage.

All of the seven soil isolates, recovered at three different time points of this study, appeared to have matching PFGE pattern with clinical isolate MN628, obtained from the child (Fig. 1). Table 3 describes the phenotypic and virulence factors detected in seven *E. coli* O157:H7 isolates retrieved from the soil samples. The isolate from the case patient and all of the soil isolates had the genes for both Shiga-like toxins (*stx*1, *stx*2), were sorbitol negative and carried other virulence factors such as intimin (*eae*A) and enterohaemolysin (*ehx*A). All isolates had typical H7 flagellar serotype.

Discussion

Escherichia coli O157:H7 is a foodborne pathogen, and its natural reservoir is ruminants that carry this pathogen asymptomatically in their gastrointestinal tract (Blanco *et al.* 1996; Hancock *et al.* 1997). Cattle typically excrete this organism in their faeces, and cattle manure is a major concern for transmission as it can be an environmental contamination source. Survival of *E. coli* O157:H7 in agricultural soil amended with contaminated cattle manure has been reported in the literature (Johannessen *et al.* 2004). A recent report suggested that hand-to-mouth contact resulted in *E. coli* O157 infections causing haemolytic uraemic syndrome in children playing in the fields contaminated with pathogen-containing manure (Grif *et al.* 2005). In this study, we documented the exposure of a child to contaminated garden soil resulting in a clinical *E. coli* O157:H7 infection.

Table 3 Characterization of isolates of *Escherichia coli* O157:H7 obtained from the patient and the garden soil samples

	Phenotype			Virulence factor		
Isolate	O157	H7	Sorbitol	*ehx*A	*stx*	*eae*A
MN628	+	+	−	+	1, 2	+
19-1*	+	+	−	+	1, 2	+
19-2	+	+	−	+	1, 2	+
19-3	+	+	−	+	1, 2	+
19-4	+	+	−	+	1, 2	+
42-2	+	+	−	+	1, 2	+
42-4	+	+	−	+	1, 2	+
69-4	+	+	−	+	1, 2	+

*Designation of soil isolates was given by the collection day and the plot number (day-plot number).

The PFGE band patterns for the case patient and soil isolates of *E. coli* O157:H7 matched when using one restriction enzyme. On the basis of this finding, all of the isolates appeared to be of the same strain (Fig. 1). This result and the epidemiological link confirmed the source of infection. There are some reports that suggest the use of more than one restriction enzyme for PFGE (Davis *et al.* 2003). However, the PulseNet standard method still uses a single enzyme, and in most cases this is sufficient to discriminate among isolates when epidemiological data are associated with the investigation of foodborne illness, farm environment or cattle herd implicated in human cases. In this case, the most likely route of infection was hand to mouth, which took place when the child was playing in the garden plots, spread with untreated cattle manure a day earlier. Our study provides the first documented evidence of the potential risk of using untreated animal manure for amending residential garden soil.

Survival of *E. coli* O157:H7 in soil has been documented by numerous reports, where soil samples were amended with animal manure or composts that were inoculated with laboratory-grown pathogenic strains. *Escherichia coli* O157:H7 has been reported to survive in soil for more than 200 days at ambient conditions and 500 days in frozen samples (Gagliardi and Karns 2002; Islam *et al.* 2004). The effect of lower temperature on enhancing survival has been observed by other researchers. The viability of *E. coli* O157:H7 in manure was increased 20 times when incubated at 5°C temperature, compared to that at 37°C (Wang *et al.* 1996; Himathongkham *et al.* 1999). In another study, when inoculated livestock wastes were spread on agricultural soil and left on the surface, the viable count declined more rapidly compared with treatments in which the manure was mixed with the soil (Maule 2000; Hutchison *et al.* 2004). All of these studies, however, utilized laboratory-grown strains inoculated into soil or manure samples.

One previously documented case of environmental survival of naturally occurring *E. coli* O157:H7 in soil was related to an outbreak of gastroenteritis among participants in a boys scout camp (Strachan *et al.* 2001; Howie *et al.* 2003). In that outbreak, 20 boys developed gastroenteritis symptoms after camping on a pasture field previously used for grazing sheep. Investigations of the field reported that a low level of 1·8 log CFU g^{-1} of *E. coli* O157:H7 was initially detected in the soil, but after 3 weeks from the onset of symptoms, the pathogen could no longer be detected (Strachan *et al.* 2001). This decline was consistent with the findings of the present study, where reduction by 1·5 log CFU g^{-1} in the *E. coli* O157:H7 count was observed in 23 days after the onset of gastroenteritis symptoms in the patient. As the initial level of contamination in our case was greater compared with the previous report, detectable levels of the pathogen persisted in the garden soil for at least 69 days.

In two recent publications, the survival of commensal *E. coli* in naturally contaminated soil amended with untreated manure was studied. *Escherichia coli* counts consistently declined by 2·5–3·0 log CFU g^{-1} within initial 50–90 days after the application of raw manure. However, low levels of the faecal indicator were detected in manure-amended soil for up to 170 days (Ingham *et al.* 2004, 2005). The fast rate of decline of generic *E. coli* within first 90 days of manure application was consistent with the finding of the present study, where *E. coli* O157:H7 counts declined by 3·4 log CFU g^{-1} in the garden soil in 92 days after the application of raw manure.

The survival of *E. coli* O157:H7 in soil and manure at temperatures below 10°C has only been reported using strains cultivated in laboratory (Wang *et al.* 1996; Himathongkham *et al.* 1999; Jiang *et al.* 2002). In one of the first papers that investigated the survival of this pathogen in manure, Wang *et al.* (1996) reported that when cattle manure was mixed with 5 log CFU g^{-1} of *E. coli* O157:H7, this organism could still be detected after 70 days. Studies using manure slurry samples inoculated with 6–8 log CFU g^{-1} suggested that the pathogen could survive for more than 120 days at 4°C (Himathongkham *et al.* 1999). In the same paper, it was noted, however, that in cattle manure the survival rate was almost twofold greater at 20°C when compared with 4°C. In a more recent paper, it was reported that *E. coli* O157:H7 can remain viable for more than 70 days in soil at 5°C and survived for more than 200 days at 15°C and 21°C (Jiang *et al.* 2002). Our findings appeared to be consistent with the latter two reports. In our study, survival at low temperature was markedly lower compared with the survival at ambient temperature, about 12–15°C during May and early June in Minnesota. At refrigerated temperature, the naturally present *E. coli* O157:H7 died at an estimated rate of 1 log CFU every 40–50 h. This difference between our findings and those studies involving inoculation with laboratory-grown cultures needs further examination.

The significance of the present study is that the soil samples were naturally contaminated and that the particular strain of *E. coli* O157:H7 present in the soil had caused a laboratory-confirmed human infection. A recent publication described a cluster of diarrhoea cases related to naturally contaminated soil with *E. coli* O157:H7 (Grif *et al.* 2005). In that report, two children contracted infection after playing on fields that were either spread with raw manure or were contaminated by runoff water from a nearby cattle farm. In addition, the survival of the pathogen was monitored in the patients and their family members but not in the contaminated soil. In the present study, among the four family members only the 2-year-old

child contracted diarrhoea and was positive for *E. coli* O157:H7. Thanks to the cooperation of the child's parents, we were able to monitor the pathogenic strain in naturally contaminated garden plots after the application of raw cattle manure in those plots. The pathogenic count declined to a nondetectable level in all the plots within 92 days after application of untreated manure (Table 3).

This study is the first documentation of a sporadic case of *E. coli* O157:H7 infection contracted by environmental exposure to contaminated residential garden plots amended with raw cattle manure. Cattle were never present in the garden, but cattle manure was used as the fertilizer in the vegetable plots, which probably led to the infection of the child by hand-to-mouth contact. In a similar case, several family members at a small farm were infected by *E. coli* O157:H7 after consuming vegetables grown in their garden amended with cattle manure (Cieslak *et al.* 1993). Composting or ageing can reduce pathogenic counts in animal manure significantly, rendering it safe to be used as fertilizer for cultivation (Lung *et al.* 2000). This report stresses the importance of using only composted materials as organic fertilizers and avoiding the application of raw manure onto residential garden soil.

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