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Escherichia coli O157:H7 diarrhoea associated with well water and infected cattle on an Ontario farm

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SUMMARY

A 16-month old female child living on an Ontario dairy farm was taken to hospital suffering from bloody diarrhoea. *Escherichia coli* O157:H7 was isolated from her stool. Initial tests of well water samples were negative for *E. coli* by standard methods but culture of selected coliform colonies on sorbitol-MacConkey agar led to isolation of *E. coli* O157:H7. *E. coli* O157:H7 was also isolated from 63% of cattle on the farm. The *E. coli* O157:H7 isolates from the child, the water and the cattle were phage type 14, produced verotoxins 1 and 2, and were highly related on analysis by pulsed field gel electrophoresis. The child did not have known direct contact with the cattle and did not consume unpasteurized milk. Hydrogeological investigation revealed the design and location of the well would allow manure-contaminated surface water to flow into the well. This investigation demonstrates that cattle farm well water is a potential source of *E. coli* O157:H7 which may not be identified by standard screening for *E. coli* in water.

INTRODUCTION

Escherichia coli O157:H7 is an important cause of bloody diarrhoea in humans. Infection may progress to the haemolytic-uraemic syndrome [1, 2]. Transmission of this organism is frequently associated with consumption of contaminated foods (particularly undercooked minced beef and unpasteurized milk) and less often with contaminated water and person-to-person transmission [2]. Because cattle are a reservoir of *E. coli* O157:H7 and other verotoxin-producing *E. coli* [3–5], exposure may also occur in the farm environment. Transmission of *E. coli* O157:H7 to a child exposed to infected calves has been reported [6]. In this report, we describe a farm where not only cattle but also the farm water supply were possible sources of *E. coli* O157:H7 causing bloody diarrhoea in a young child.

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THE CASE AND THE INVESTIGATION

On 4 June 1995, *E. coli* O157:H7 was isolated from the stool of a 16-month old girl hospitalized because of bloody diarrhoea and abdominal cramps. The child, the youngest of three children living on a dairy farm, was the only ill family member. She did not consume raw milk or ground beef and had no direct contact with the cattle or barns. She was in frequent

Table 1. *Presence of coliforms and* E. coli *in well water samples*

Sample number*	Number of coliforms per 100 ml	Number of *E. coli* per 100 ml	Number of presumptive *E. coli* O157 per 100 ml
1 (IF)	50	0	4
1A (GPF)	56	0	0
2 (IF)	6	3	0
2A (GPF)	0	0	0
3 (IF)	4	4	0
3A (GPF)	O/G†	O/G†	NA‡
4 (IF)	1	1	0
4A (GPF)	1	0	0

* Samples from the infant's farm (IF) and the grandparents' farm (GPF) submitted together on four occasions between 7 June and 4 July 1995.
† O/G, overgrown.
‡ NA, Not applicable because of overgrowth of non-coliforms.

contact with an orphaned pet lamb. On 7 June 1995, water samples from the shallow dug well supplying water to the farmhouse and the barns and from the grandparents' adjacent home (which shared the same groundwater), were submitted to the Hamilton Public Health laboratory (PHL). The samples were tested according to Ontario Ministry of Health procedures [7, 8] for total coliforms and total *E. coli*. Briefly, 100 ml samples were filtered under vacuum through 0·45 μm cellulose acetate membrane filters. One filter was incubated at 37 °C for 20 ± 2 h on m-Endo LES (Difco Laboratories, Detroit, MI) for quantitation of coliforms [7, 8] and another was incubated at 44·5 °C for 20 ± 2 h on FC-BCIG medium [7] consisting of FC agar base (Difco) containing 5-bromo-4-chloro-3-indoxyl-β-D-glucuronic acid (BCIG, Diagnostic Chemicals Ltd, Charlottetown, P.E.I., Canada). There was no evidence of growth on the FC-BCIG plate, indicating an absence of *E. coli* (Table 1). Because of the child's illness 10 colonies from the coliform membrane were subcultured onto sorbitol-MacConkey (SMAC) agar [2] for detection of non-sorbitol-fermenting *E. coli*. Four of the 10 subcultured colonies did not ferment sorbitol and were presumptively identified as *E. coli* O157:H7 by a slide agglutination test with anti-*E. coli* O157 antiserum (Toronto PHL). Confirmation and additional typing were carried out by the Toronto PHL. Three further sets of water samples collected over the ensuing month were tested similarly. Some contained sorbitol-fermenting *E. coli* but were negative for *E. coli* O157:H7 (Table 1). Whether or not *E. coli* colonies were detected on FC–BCIG plates, representative colonies from coliform plates were also screened using SMAC to see if *E. coli* O157:H7 was present (to increase sensitivity of detection).

On 13 June 1995, a veterinarian from the Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA) collected faecal pellets from the lamb and rectal swabs from the cattle for testing at the Health of Animals Laboratory, Guelph. The samples were cultured overnight at 37 °C in modified *Escherichia coli* (mEC) broth containing novobiocin (20 mg/l) [9]. A portion (1 ml) of the culture was processed by immunocapture on magnetic beads coated with antibodies to *E. coli* O157:H7 (Dynal, Oslo, Norway) [9]. After magnetic separation and washing, the beads were incubated in mEC broth for 6 h and then cultured on SMAC agar containing cefixime (0·05 mg/l) and potassium tellurite [10] overnight at 37 °C. Non-sorbitol-fermenting colonies on this medium were tested for VT production by a Vero cell cytotoxicity assay (VCA) [1, 3]. Filtrates from initial mEC broth cultures were also tested by the VCA, and positive cultures were processed for isolation of VTEC [3]. VT-positive isolates obtained by immunocapture or from the initial mEC broth were serotyped.

E. coli O157:H7 was isolated from 25 of 45 calves (56%) and 34 of 50 adult cattle (68%) by the immunocapture method. Testing of the initial bovine faecal cultures by VCA resulted in isolation of seven other serotypes of VTEC, namely O22:H8, O26:H11, O84:NM, O111:H8, O116:H21, O136:H12, and OR:H11. *E. coli* O157:H7 or other VTEC were not isolated from the lamb.

Subsequent characterization of *E. coli* O157:H7 isolates from the child, the well water and from eight cattle indicated they were very closely related. Phage typing [11] at the National Laboratory for Enteric Pathogens revealed all the tested isolates were phage type 14 and by polymerase chain reaction amplification [12, 13] they all carried genes for VT1 and VT2. In addition, the DNA profiles obtained by pulsed field gel electrophoresis analysis of the isolates, as described by Barrett et al. [14] with minor modifications, were identical except for a slight difference in the size of one band in the isolate from the child.

In view of the evidence for a common source of infection on the farm, a hydrogeological inspection of the septic system, well connections, manure lagoon, and dormant wells was conducted on 3 August 1995.

The farm well head was defective and located below the surface grade, allowing manure-contaminated surface water to flow into the well. Another concern was an open connection between a dormant barn cistern and the farm well. Concentrations of sodium (37–73 mg/l) chloride (< 0·1–1·7 mg/l) and nitrate (23–85 mg/l) in water samples taken from several sites at this time were within normal ranges [15], suggesting there was no direct contamination of the water supply from septic system or manure lagoon.

DISCUSSION

The results of this investigation strongly implicate the farm as the source of the child's infection. The *E. coli* O157:H7 isolated from the cattle and the farm water supply were the same toxin type and phage type as the isolate from the child, and isolates from all three sources were genotypically highly related. Whereas previous human infections in farm settings have been linked to contact with infected cattle manure or consumption of raw milk [1, 2, 6], this child reportedly had no direct contact with cattle, did not visit the barns nor consume raw milk. The organism may have been transmitted to the house on clothing, shoes or hands of persons working with the cattle. However, the isolation of a highly related strain of *E. coli* O157:H7 from the well water soon after the onset of illness implicates the drinking water as a possible source.

Cattle were the most probable source of *E. coli* O157:H7 on the farm. They are a known reservoir of *E. coli* O157:H7 [3–5], and spread of the organism from cattle to the water supply was possible due to the design and subgrade location of the shallow dug well. Contamination of the well water may also have increased exposure of cattle on the farm. The proportion of *E. coli* O157:H7-infected cows (68%) and calves (56%) was far higher than in several surveys [2–5] and in two herds associated with human infection [6, 16]. Circulation of the organism between cattle and the water supply would provide an on-going risk of exposure for the farm family and the cattle.

Although water has been associated with community outbreaks of *E. coli* O157:H7 infection [17, 18], we are unaware of previous reports linking contaminated well water with farm-associated *E. coli* O157:H7 illness. Water from shallow dug wells has been identified as a risk factor for other intestinal infections [19]. The lack of reports of isolation of *E. coli* O157:H7 from the farm well water may, in part, reflect that the standard methods used by many laboratories, including the Ontario Ministry of Health Public Health Laboratories, for detection of *E. coli* in well water do not detect *E. coli* O157:H7. Differentiation of *E. coli* relies on the fact that most *E. coli* possess the enzyme β-glucuronidase [7]. However, *E. coli* O157:H7 does not have this enzyme activity [2] and therefore does not produce the characteristic coloured colonies. In this investigation, *E. coli* O157:H7, though not expected to show the characteristic colour, did not grow at all on FC–BCIG medium. The method also relies upon growth at 44·5 °C, a temperature at which *E. coli* O157:H7 may not grow well [2]. It is possible the *E. coli* O157:H7 in the initial water sample were stressed and therefore unable to grow on FC–BCIG medium at 44·5 °C. Once recovered and subcultured, however, the isolates from the well water, the child and 15 cattle isolates grew as typical *E. coli* O157:H7 colonies under these conditions. Failure to isolate the organism from subsequent water samples despite the screening of colonies from coliform plates as well as FC–BCIG suggests that contamination of the water was transient or intermittent. Despite the fact that the well was treated after the initial isolation, coliforms and *E. coli* were isolated in some subsequent samples.

This investigation provides further evidence that the dairy farm environment is a source of human exposure to *E. coli* O157:H7 and other VTEC, and draws attention to the possible role of farm well water in the epidemiology of human and bovine *E. coli* O157:H7 infections in this setting. The findings also emphasize that some standard screening methods for microbiological testing of well water will not detect *E. coli* O157:H7. When diarrhoeal illness epidemiologically related to water is suspected or a reservoir of *E. coli* O157:H7 exists, alternative methods must be used to detect this organism.

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