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Infection with verocytotoxin-producing *Escherichia coli* O157 during a visit to an inner city open farm

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SUMMARY

Two cases of *Escherichia coli* O157 infection occurred in children after visiting an inner city open farm. Subsequently faecal samples collected from animal pens and samples of composted mixed animal manure and vegetable waste were examined for *E. coli* O157 by enrichment culture, immunomagnetic separation and culture of magnetic beads to cefixime tellurite sorbitol MacConkey agar. Strains of *E. coli* O157 were characterized by hybridization with DNA probes for VT1, VT2 and *eaeA*, plasmid profile analysis, phage typing and pulsed field gel electrophoresis (PFGE). Verocytotoxin-producing *E. coli* O157 strains were isolated from faecal samples from a cow, a horse, 3 breeds of pigs, 2 breeds of sheep and 2 breeds of goats and from 2 samples of compost which had been processed for 3 months. All strains were phage type 21, hybridized with probes for VT2 and *eaeA* but not with one for VT1, harboured 92 and 2 kb plasmids and gave indistinguishable banding patterns with PFGE. Although only two culture-confirmed cases of infection had been identified, the farm had over 100000 visitors per year and so it was closed as a precaution both to allow a thorough investigation and to prevent further cases. The investigation identified many factors which may have contributed to transmission of *E. coli* O157 infection. Most of these were readily resolved by appropriate corrective measures and as there were no further cases associated with the farm during the ensuing 4 weeks it then re-opened. These cases highlight the risk, especially to young children, of acquiring zoonotic infections during visits to open farms and emphasize the need for adequate guidance and supervision before and during such visits.

INTRODUCTION

Verocytotoxin-producing (VT^+) *E. coli* (VTEC) cause haemorrhagic colitis (HC), haemolytic-uraemic syndrome (HUS) and occasionally mild non-bloody diarrhoea in man; infections may be asymptomatic [1]. In the UK, VT^+ *E. coli* O157, the most common serogroup associated with illness in man, has been isolated from cattle [2, 3]. Beef and beef products, milk and milk products, and vegetables or fruit contaminated with animal manure have been identified as sources of human infection with the organism [4–7]. Person-to-person transmission of *E. coli* O157 and transmission by direct contact with animals or animal manure have also been reported [8–10].

In August 1997, *E. coli* O157 infection occurred in two children who independently visited an inner city open farm in Sheffield. This paper describes subsequent investigations at the farm and the problems encountered.

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MATERIALS AND METHODS

The cases

The first case was a 3-year-old boy who developed bloody diarrhoea, vomiting and abdominal pain on 30 July 1997 and was admitted to hospital the next day. Faecal culture yielded *E. coli* O157. The father developed similar symptoms on 29 July but *E. coli* O157 was not isolated from a faecal sample submitted on 8 August. No other cases occurred in the household or in close contacts.

The second case was a 4-year-old girl who developed diarrhoea and abdominal pain on 30 July. Again faecal culture yielded *E. coli* O157 and no other cases occurred in the household or in close contacts.

All three cases had visited the same inner city open farm on 26 July. The second child only had attended a children's birthday party at the farm cafeteria, whereas all three cases had had close contact with the farm animals. The farm was the only common link that was found between the cases on questioning. A further 30 persons were identified as having attended the birthday party; none had developed symptoms subsequently. During the next 3 days, faecal samples were obtained for microbiological testing from all of birthday party attendees and all of the farm staff.

It was not possible to trace other visitors on that day as the farm did not keep records of attendance.

The farm

The farm is situated in an inner city area of Sheffield and is surrounded by residential houses. It is open to the public during normal visiting hours. However, children regularly play on the premises outside these times as the position of the farm precludes construction of a secure perimeter barrier. The farm is a centre for school educational visits and has about 100000 visitors per annum. As well as 19 full-time paid staff, the farm also employs adult volunteers and operates a work experience scheme in conjunction with a local centre for people with learning difficulties.

The livestock at the farm included a cow (Irish Moiled), a horse (Exmoor Pony), 3 breeds of pigs (2 Large Blacks, 1 Large White/Landrace Cross and 1 Vietnamese Pot-Bellied), 2 breeds of sheep (3 Leicester Longwool and 3 Soay) and 2 breeds of goats (4 Saanen and 2 Angorra); each breed, but not each animal, was penned separately. The livestock also included numerous rabbits, waterfowl and other birds of various breeds.

The farm was visited by Environmental Health Officers on 6 August and any apparently fresh faecal samples were collected from the animal pens. The number of samples collected from each animal pen were cow 3, horse 1, pigs (Large Black) 5, pigs (Large White/Landrace Cross) 1, pigs Vietnamese Pot-Bellied 1, sheep (Leicester Longwool) 10, sheep (Soay) 10, goats (Saanen) 10, goats (Angorra) 11, rabbits 10, waterfowl 28 and other birds 10. In addition two samples of part finished compost and two samples of finished compost were collected: the compost was made using mixed animal manure and vegetable waste.

On 13 August a Veterinary Investigations Officer visited the farm and collected rectal swabs from all the large animals. All samples were submitted to Sheffield Public Health Laboratory for microbiological testing.

The farm was visited by Environmental Health Officers and a Microbiologist on 9 and 11 August and by a Veterinary Investigations Officer on 13 August; all visits were to identify any problem(s) which may have contributed to the human cases of infection.

Isolation and characterization of *E. coli* O157

Human faecal samples were inoculated directly onto cefixime tellurite sorbitol MacConkey agar (CT–SMAC) [11] and incubated overnight at 37 °C.

Approximately 0·5 g of human or animal faecal samples or the entire animal rectal swab were placed in 5 ml of buffered peptone water (Oxoid – CM509) supplemented with vancomycin 8 mg/l, cefixime 0.05 mg/l, and cefsulodin 10 mg/l (BPW–VCC) [2] and 25 g samples of compost were added to 225 ml of BPW–VCC. After mixing, broths were incubated at 37 °C for 6 h and *E. coli* O157 was concentrated from 1 ml of broth by a previously described immunomagnetic separation technique [12]. Washed and separated beads were inoculated on to CT–SMAC medium and incubated overnight at 37 °C.

Sorbitol non-fermenting colonies were tested for agglutination with a latex test kit (Oxoid – DR622) for detecting *E. coli* O157. Isolates that gave a positive latex test result were confirmed as *E. coli* by biochemical tests (Crystal ID, Becton Dickinson) and confirmed as serogroup O157 by agglutination to titre with antiserum to *E. coli* O157 (Laboratory for Microbiological Reagents, Central Public Health Laboratory, London).

Presence of the VT_1, VT_2 and *eaeA* genes was

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determined by DNA hybridization and plasmid analysis performed as described previously [3].

Pulsed field gel electrophoresis (PFGE) was performed as described by Thomson-Carter and colleagues [13]. Bacterial cells suspended in chromosomal grade agarose (Bio–Rad) plugs were sequentially treated with lysozyme, RNAse, proteinase K, phenylmethylsulphonylfluoride and Tris–EDTA buffer before being digested with *Xba*I 30 U at 37 °C for 6 h. PFGE was performed in a CHEF DR-II apparatus (Bio–Rad) with 1% agarose gels in Tris-borate-EDTA buffer. Gels were electrophoresed for 22 h at 14 °C at a constant 6 volts/cm and with pulse times of 5–50 s with linear ramping. Gels were stained with ethidium bromide and visualized on an ultraviolet light transilluminator.

E. coli O157 isolates were phage typed by the Laboratory for Enteric Pathogens, Central Public Health Laboratory using the method of Khakhria and colleagues [14].

RESULTS

E. coli O157 was not isolated from faecal samples from any of the asymptomatic attendees of the birthday party or from the farm staff.

E. coli O157 was isolated from the faecal samples collected on 8 August from the pens of the cow (1 sample), Large Black pigs (1 of 5 samples), Large White/Landrace Cross pig (1 sample), Vietnamese Pot-Bellied pig (1 sample), Leicester Longwool sheep (7 of 10 samples), Soay sheep (8 of 10 samples), Saanen goats (3 of 10 samples), and Angorra goats (4 of 11 samples). *E. coli* O157 was isolated from rectal swabs taken on 13 August from the cow, the Vietnamese Pot-Bellied pig, 1 Leicester Longwool sheep, 1 Saanen goat and 1 Angorra goat. The organism was also isolated from two samples of finished compost but not from two samples of part-finished compost. *E. coli* O157 was not isolated from any of the samples collected from rabbits, waterfowl and other birds.

All isolates of *E. coli* O157 were phage type 21, hybridized with probes for *eaeA* and VT_2 but not with one for VT_1, harboured a 92 kb and a 2 kb plasmid and gave indistinguishable banding patterns by PFGE.

During visits to the farm the following were identified as potential factors contributing to the cases of infection acquired from the farm: (1) the overall design of the farm allowed very close contact between visitors and animals, (2) the open nature of the site with more than one uncontrolled entry point meant that it was impossible to restrict the entry of people outside normal hours, (3) the walkways for visitors were contaminated with faecal slurry, (4) the animals were in very close proximity to one another and shared water and food troughs, (5) untreated goats' milk was fed to the other large animals, (6) there was completely free movement of staff from the main stable to the rest of the farm, (7) the main stable housing all the large animals was open to the public and was also used as a thoroughfare and became very crowded at busy times or during inclement weather, (8) access to the composting area and to the area for storage of feedstuffs was not restricted, (9) the compost was sold to the public from the garden centre, (10) vegetables grown in the compost, or in soil treated with the compost, were allowed to be picked for home consumption, (11) toilet facilities were insufficient in number and lacked regulated hot water, soap dispensers, wash basins accessible to young children and adequate notices advising hand washing, (12) in the cafeteria there was no dedicated hand washing facility for the public entering from the farm, farm workers had access to the kitchen, there was no suitable insect control which allowed flies from the farm to enter food preparation areas, and (13) a designated play area allowed children to contaminate a range of toys with soiled footwear or dirty hands.

DISCUSSION

Of the 67 cases of human infection with *E. coli* O157 that occurred in the Sheffield area during 1997, only a further 6 were of the same phage type, toxin genotype and plasmid profile as the strains associated with the farm and none of these cases had a history of visiting the farm at any time during the previous 4 weeks. Although only two culture-confirmed cases of infection were associated with the farm, a thorough investigation was thought necessary in view of the potential risk of infection to over 100000 visitors per year. *E. coli* O157 has a low infective dose [1, 15] and infection from direct contact with animals has been documented previously [9]. These cases highlight the risk, especially to young children, of acquiring zoonotic infections during visits to open farms; such infections have been documented previously [16, 17].

The farm management agreed to close the farm on 8 August to allow a full review of procedures. During visits to the farm on 9, 11 and 13 August numerous

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factors were identified which had the potential to contribute to acquiring infection from the farm; many of these had been identified in previously published advisory information [18–20]. Several corrective measures were introduced immediately: (1) the main stable block was closed to the public, (2) improved multi-lingual signs, informing visitors of hygiene precautions to be taken, were erected at all entry points to the farm, (3) more rigorous cleaning and disinfection regimes were introduced for the stables, feed and water troughs and footpaths, (4) individual feed and water troughs were provided for each animal pen, (5) goats' milk was not fed to other animals, (6) all staff and volunteers received further training in hygiene measures necessary on the farm and disinfectant footbaths were installed for use when entering or leaving the main stable block, (7) access to the composting areas was restricted by erecting barriers carrying large 'keep off' notices, (8) the minimum processing time of the compost was increased from 3 to 6 months, (9) toilet and hand washing facilities on the farm were upgraded, (10) hand washing facilities were provided for visitors entering the cafeteria, (11) the play area in the cafeteria was closed permanently.

Through investigation of the two cases, a number of questions were raised about the farm and its procedures which caused concern to the investigation team because of the need to take decisions on closure, re-opening and the sale of farm produce with little published guidance. These included: (1) addressing the financial difficulties and possible bankruptcy if the farm was closed for a prolonged period, (2) balancing the beneficial recreational activities offered by the farm against the continued risk of *E. coli* O157 infection, (3) whether animals found to be carrying *E. coli* O157 should be destroyed and replaced and if so whether colonization of replacement stock with the organism could be prevented, (4) whether people with learning difficulties should be allowed to work in such a potentially hazardous environment and, (5) whether the farm should continue to sell potentially contaminated salad vegetables with clear washing instructions or whether it should sell only those vegetables which were to be cooked before consumption. These issues were addressed pragmatically. There were no further cases associated with the farm during the ensuing 4 weeks and the farm re-opened on 12 September.

There were two main points of microbiological interest during investigation of the farm. *E. coli* O157 was isolated from most of the animal species. Although VT+ *E. coli* O157 has been isolated previously from cattle [2, 3], sheep [3], goats [21, 22] and a horse [23], most strains of *E. coli* O157 previously isolated from pigs have not produced VT or harboured the *eaeA* gene and have not been characteristic of those causing infection in man [3, 24, 25]. In the present study we isolated VT+, *eaeA*+ strains of *E. coli* O157, which were indistinguishable from those strains associated with the human cases, from three different breeds of pigs; although at the time of this investigation we were not aware of other published reports of the isolation of the organism for pigs, this has since been reported in both pigs at slaughter [26, 27] and farm pigs housed separately from other animals [28].

E. coli O157 strains, again indistinguishable from those isolated from the human cases, were isolated from two samples of composted mixed animal manure and vegetable waste which had been processed for 3 months, but not from two samples of part-finished compost which had been processed for only 6 weeks. The compost was sold to the public, used as a soil conditioner and fertilizer on the vegetable plots and was used as a supplement to growing media used for the production of glasshouse crops. Many of these crops were salad vegetables to be consumed uncooked and, as the farm operated a 'pick-your-own' scheme, this clearly represented a risk of infection. Although *E. coli* O157 can survive for 70–100 days at 4–8 °C in animal faeces [29–31] and may survive in manure slurry for up to 6 weeks [32], survival studies of the organism during the composting of mixed animal manure and vegetable waste has not been reported. Studies of the composting of animal manure and food biowaste separately [33–35] suggest that *E. coli* O157 should not have survived the 3 months of composting, but that if survival had occurred then the organism could again have multiplied to large numbers in the composted solids; this could explain why we found *E. coli* O157 in samples of finished compost, but not in samples of part-finished compost. Compost now sold from the farm carries a quite clear health warning and the basic hygiene precautions which should be observed during its handling.

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