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Escherichia coli O157 infection associated with a petting zoo

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SUMMARY

A young child was admitted to hospital with haemolytic-uraemic syndrome caused by infection with a Shiga toxin 2-producing strain of *Escherichia coli* (STEC) O157. Five days before he became ill, the child had visited a small petting zoo. STEC O157 strains were isolated from faecal samples from goats and sheep housed on the farm. The human and the animal isolates were indistinguishable by molecular subtyping. The petting zoo voluntarily closed temporarily to prevent further cases of infection. Two out of 11 other, randomly selected petting zoos (including one deer park) visited subsequently, tested positive. Furthermore, during the study period there was one more notification of STEC O157 infection possibly linked with a farm visit. Although STEC O157 was indeed found in the petting zoo associated with this patient, transmission through animal contact could not be confirmed because the human isolate was not available for subtyping. The case study and the results of the other on-farm investigations highlight the risk of acquiring severe zoonotic infections during visits to petting zoos.

INTRODUCTION

Shiga toxin (Stx)-producing *Escherichia coli* (STEC) O157 (STEC O157) were first recognized as human pathogens in 1982 [1]. Since then, the number of reported outbreaks and sporadic cases of infection caused by STEC O157 have increased and the organisms are now recognized as an important new zoonotic agent giving rise to serious public health concern in industrialized countries [2]. Infection with STEC O157 presents with a variety of clinical manifestations [3]. Diarrhoea is the most common clinical presentation. More severe manifestations include haemorrhagic colitis and the haemolytic-uraemic syndrome (HUS), particularly occurring at the extremes of age. An infection with STEC is the most common cause of HUS in North America and Europe, and HUS is the most common cause of acute renal failure among children in these areas. HUS develops, on average, 1 week after the onset of diarrhoea and is heralded by increasing pallor, mild jaundice, decreasing urine output, oedema, and sometimes, seizures. It occurs in about 10% of STEC O157 infections. Most patients recover with appropriate supportive therapy, but approximately 5% of

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affected patients die in the acute phase of the illness and an equal number will have severe sequelae, such as long-term renal impairment, neurologic injury, or hypertension. Many patients who regain renal function have chronic proteinuria, and some develop end-stage renal disease years or even decades later.

Cattle appear to be a major natural reservoir of STEC O157 and an important source of human infection, because human infections are frequently linked with the ingestion of undercooked ground beef and unpasteurized cow's milk [4]. STEC O157 strains have also been isolated from other domestic animals including sheep, goats, horses, pigs, geese, and turkeys [5]. However, the extent to which these animal species play a role in the epidemiology of STEC O157 infection remains to be established. Although most infections of STEC O157 in humans have been linked to exposure to a food vehicle or water, person-to-person transmission of STEC O157 and transmission by direct contact with animals or animal manure have also been reported [3].

In July 2000, STEC O157 infection occurred in a 17-month-old boy who visited a petting zoo. Here we report the results of subsequent investigations at this petting zoo and of a bacteriological study of the occurrence of STEC O157 on other petting zoos.

MATERIALS AND METHODS

The HUS case and the investigation

On 30 July 2000, a 17-month-old boy developed severe abdominal pains and slimy, bloody diarrhoea, having been listless for 2 days. He became more somnolent, pale, and his urinary output fell. He was admitted to hospital on 2 August and the clinical picture resulted in the diagnosis of haemolytic-uraemic syndrome. The renal insufficiency was treated with peritoneal dialysis. Gradually diuresis increased and the boy was discharged from hospital on 28 August. In the laboratory serum antibodies to *E. coli* O157 lipopolysaccharide were detected (Maroeska te Loo, University Hospital Nijmegen, Department of Paediatrics, Nijmegen, The Netherlands) and stool culture yielded an STEC O157 isolate (Diagnostic Laboratory of the Department of Medical Microbiology, University Hospital Amsterdam). Notification of the STEC O157 isolate prompted the Municipal Health Service to make further enquiries and trace contacts. The mother of the boy was interviewed using the standard questionnaire of the enhanced laboratory-based surveillance system of STEC O157, set up in 1999, of the National Institute of Public Health and the Environment (RIVM). The questionnaire focusses on details of the illness, travel history, food history, exposure to farm animals and pets. The boy did not consume high risk foods, such as undercooked ground beef and unpasteurized milk, but had regularly played on a farm belonging to relatives. Furthermore, he had visited a petting zoo 1 week before the onset of symptoms. He had petted the animals and had played on the ground. No other cases occurred in the household or in close contacts.

Because recent contact with animals was thought to be the most probable source of the infection, the farm of the relatives and the petting zoo were visited on 31 August and 19 September, respectively. From all adult dairy cows and calves present on the farm individual rectal swabs were taken. From the sheep apparently freshly voided, single faecal samples were collected in the pasture. The petting zoo maintained goats, sheep, chickens, guinea fowls, a peacock, pigeons, a pig, and rabbits. During the initial field investigation at the petting zoo, samples of single fresh droppings from these animals and water samples from drinking troughs were collected. At subsequent visits (6 October, 30 October, 14 November, 11 December), all goats and sheep present were sampled individually by digital rectal palpation. Microbiological investigations were undertaken to test for the presence of STEC O157 and any isolates were compared with the strain from the human case.

The occurrence of STEC O157 in petting zoos

Eleven other petting zoos and a deer park were visited to test the presence of STEC O157. While the majority of the petting zoos was selected randomly, one (petting zoo K) was visited because it was linked by the RIVM enhanced laboratory-based surveillance system with a case of bloody diarrhoea caused by STEC O157. A 21-month-old boy became ill 5 days after a visit to the petting zoo. Another petting zoo (petting zoo I) and the deer park were selected because they are owned by the same person as the petting zoo associated with the HUS case described above. At initial visits of these petting zoos, apparently fresh droppings were collected in the paddocks and pens and occasionally samples of animal drinking water and feed. If STEC O157 strains were isolated, additional sampling was done at the petting zoo in question to monitor the

Table 1. *Isolation of STEC O157 from goats and sheep sampled individually during the follow-up investigations at the petting zoo which was linked to the HUS case*

	Date of sampling*			
Animal	06-10-00	30-10-00	14-11-00	11-12-00
Goat 1	−	+	−	−
Goat 2	−	−	−	−
Goat 3	−	−	−	−
Goat 4	−	−	−	−
Goat 5	−	−	−	−
Goat 6	+	−	−	−
Goat 7	−	−	−	−
Goat 8	+	−	−	−
Goat 9	−	−	−	−
Goat 10	−	−	−	−
Goat 11	ND	−	−	−
Sheep 1	−	−	+	−
Sheep 2	−	+	−	−
Sheep 3	−	−	−	−
Sheep 4	−	−	+	−

* +, positive; −, negative; ND, not done.

prevalence and persistence of STEC O157. For this purpose animals and those present in the positive paddock and/or pen were sampled individually by digital rectal palpation or by collecting freshly voided faecal samples, and from the remaining animals present on the farm fresh environmental droppings were collected.

Isolation and characterization of STEC O157

At the laboratory, samples were either analysed immediately or held at 4 °C for no longer than 72 h before analysis. Samples were enriched in modified tryptone soy broth containing novobiocin (20 mg/l) (Sigma Chemical Co., St. Louis, MO) (mTSB) [6] for a maximum of 20 h at 41 °C, and subjected to two commercially available screening methods for the presence of STEC O157, an automated immunoconcentration (ICE) system (VIDAS-ICE) (bioMérieux, Lyon, France) and an immunomagnetic separation and concentration (IMS) assay (Dynabeads anti *E. coli* O157) (Dynal, Oslo, Norway), before subculture onto sorbitol-MacConkey agar (SMAC) (Oxoid Ltd., Basingstoke, UK) supplemented with cefixime (0·05 mg/l) and potassium tellurite (2·5 mg/l) (Oxoid) (CT-SMAC) [7] and CHROMagar™ O157 (CHROMagar, Paris, France) supplemented with cefixime (0·05 mg/l) and potassium tellurite (2·5 mg/l) (CT-CHROM). The ICE and IMS procedures were performed according to the manufacturer's instructions. After incubation at 37 °C for 18–20 h, typical colonies (non-sorbitol-fermenting colonies on CT-SMAC; β-glucuronidase-negative colonies on CT-CHROM) were selected and screened (up to eight per sample) for lactose fermentation on Levine's eosin methylene blue agar (Oxoid) and the absence of β-glucuronidase and sorbitol fermentation on SMAC containing 4-methylumbelliferyl-β-D-glucuronide (0·1 g/l) (Sigma) [8]. Isolates thus selected were tested by latex-agglutination with an *E. coli* O157 latex test kit (Murex Biotech Ltd., Central Road, Temple Hill, Dartford, Kent, UK). Latex-agglutinating isolates were confirmed biochemically as *E. coli* by an API 20E test (bioMérieux), and subjected to a PCR assay specific for a portion of the *rfb* (O-antigen-encoding) region of *E. coli* O157 [9].

All isolates confirmed to be *E. coli* O157 were subjected to a multiplex PCR assay to determine the presence of *stx* genes (stx_1 and/or stx_2) and the *E. coli* attaching-and-effacing (*eae*) gene [10]. All *E. coli* O157 isolates were additionally tested for the hly_{EHEC} gene [11], the $fliC_{\mathrm{h7}}$ gene [12] and subjected to the STEC O157-specific SZ-PCR assay [13]. Phage typing was done at the Laboratory of Enteric Pathogens of the Central Public Health Laboratory in London (United Kingdom). The pulsed-field gel electrophoresis (PFGE) technique of contour-clamped homogeneous electric fields (CHEF) was used for genomic typing of

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the isolates [14]. Genomic DNAs were digested in agarose plugs with *Xba*I (10 U) (Roche, Mannheim, Germany). The resulting fragments were resolved by CHEF-PFGE with a CHEF DR-III apparatus (Bio-Rad Laboratories, Richmond, California) at a constant voltage of 200 V for 20 h at 13 °C and a linearly ramped pulse time of 2·2–54·2 sec.

RESULTS

The HUS case and the investigation

Faecal samples from dairy cows ($n = 44$), calves ($n = 7$), and sheep (15 droppings) obtained from the farm of the patient's relatives were all found to be negative for STEC O157. STEC O157 strains were isolated from 8 of 27 droppings from goats and/or sheep collected during the first field investigation at the petting zoo. STEC O157 strains were not detected in faeces from the pig ($n = 1$), droppings from birds ($n = 2$), or the samples of animal drinking water ($n = 2$). Table 1 shows the results of the four revisits at which the goats and sheep were all sampled individually. A comparison of the STEC O157 isolates by Stx and phage type, and PFGE demonstrated that all animal isolates were indistinguishable from each other and from the human isolate. All strains were positive for stx_2 only and reacted with the typing phages but did not conform to a recognized pattern ('reacts but does not conform' (RDNC)). The strains were further characterized as: $eae+$, $hly_{\mathrm{EHEC}}+$, $fliC_{\mathrm{h7}}+$ and showed positive results in the SZ-PCR assay. Additional samples collected were: a composite sample of both pig droppings and rabbit droppings at the first revisit, and at the last revisit single droppings from goats ($n = 2$) and sheep ($n = 2$) and a composite sample of bird faeces. No STEC O157 strains were isolated from these samples.

Actions immediately taken following the isolation of STEC O157 were the following. The petting zoo was closed voluntarily to visitors and animals with confirmed STEC O157 infections were isolated in the shed. Additionally, the owner put in place additional recommended general hygienic practices.

The occurrence of STEC O157 in petting zoos

Samples collected in 3 of the 12 petting zoos visited (including the deer park) tested positive for STEC O157 (Table 2). STEC O157 were isolated from droppings collected from goats, turkeys, a heifer, and

Table 2. *Isolation of STEC O157 from faecal samples collected in petting zoos*

Farm	Date of sampling	Number of fresh environmental samples tested	Nature of specimen								STEC O157 isolated from
			Goats	Sheep	Goats and/or sheep	Birds	Cattle	Deer	Pigs	Horses/ pony's/ donkeys	Rabbits/ guinea-pigs	
A	09-10-00	40	20			12	3		1	3	1	2/20 goats and 2*/12 birds
B	12-10-00	29	7	7		2	1			11	1	0
C	12-10-00	33	10	12	3	2	1		2	1	1	0
D	31-10-00	38	7	13		4			1	2	3	0
E	31-10-00	34	7	20		1	1		1	3	1	0
F	03-11-00	22	5	8		1	5		2		1	0
G	20-11-00	20	3	4		6	1		1	1	1	0
H	20-11-00	21	6		5	4	1		1	2	1	0
I	27-11-00	17	11	3	1	1						0
J†	27-11-00	9				1		8				0
K	21-12-00	21	15	3			1		1	1		1/1 heifer
L	03-01-01	24	3		7	3		8	1	2		1/8 deer

* Turkeys; † Deerpark.

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a fallow deer. The petting zoo and the deer park owned by the same person as the petting zoo associated with the HUS case described above both tested negative.

On petting zoo A, the STEC O157 strains were isolated from 1 of the 2 goat paddocks. On 23 October 2000, the goats ($n = 10$) in the positive paddock were all individually sampled by rectal palpation or freshly voided individual samples of faeces were collected, to identify positive animals. Two (20%) of the goats were found to be positive. In addition samples of apparently fresh goat droppings were collected randomly in the positive paddock as well as pooled samples of faeces from birds that shared the same paddock (chickens, guinea fowls, geese, turkeys, and ducks). STEC O157 strains were not isolated from these fresh environmental faeces. Droppings collected in the other goat paddock and at other sites of the petting zoo were found to be negative again. The petting zoo subsequently was visited another five times (22 November 2000, 12 December 2000, 19 December 2000, 9 January 2001, 19 February 2001). The results of the individual samplings of the goats in the positive paddock were as follows: 100% ($n = 10$), 33% ($n = 9$), 22% ($n = 9$), 0% ($n = 9$), and 0% ($n = 8$) positive, respectively. The results of fresh environmental samples collected in addition were: 23% ($n = 26$) (3/5 goat droppings and 3/9 turkey droppings), 4·5% ($n = 44$) (2/9 goat droppings), 0% ($n = 0$), 0% ($n = 30$), and 0% ($n = 9$) positive, respectively. The infection remained in the paddock initially found positive, but after the fourth revisit there were no further isolations of STEC O157 from any of the samples collected. All isolates were characterized as: stx_2+, $eae+$, $hly_{\mathrm{EHEC}}+$, $fliC_{\mathrm{h7}}+$ and showed positive results in the SZ-PCR assay. They were of PT54 and all showed identical PFGE patterns.

As soon as the results of microbiological testing were definite the positive heifer of petting zoo K was segregated from public access. On 23 January 2001 the petting zoo was visited again. All faecal samples ($n = 13$) collected were found to be negative. The heifer isolate was typed as PT54, stx_2+, $eae+$, $hly_{\mathrm{EHEC}}+$, $fliC_{\mathrm{h7}}+$, gave a positive result in the SZ-PCR assay and showed a unique PFGE pattern. Unfortunately, the STEC O157 isolate from the boy who had visited this petting zoo 5 days before he became ill was not available for subtyping.

Petting zoo L was revisited on 30 January 2001. This time, STEC O157 was isolated from a fresh sheep dropping ($n = 5$). The remaining 19 fresh environmental samples collected were found to be negative. A few days after our first visit the fence between the two adjoining deer paddocks was closed. In the one paddock all deer were kept and the sheep were moved into the other. While both the deer and the sheep isolate were typed as PT54, stx_2+, $eae+$, $hly_{\mathrm{EHEC}}+$, $fliC_{\mathrm{h7}}+$, and gave a positive result in the SZ-PCR assay, their PFGE patterns differed by seven bands. No specific measures were taken at the farm. Visitors do not have access to the paddocks and the fences prevent close contact between visitors and animals.

Results of PFGE genotyping showed that isolates obtained from different farms were all of distinct STEC O157 strain types. Upon comparison of the PFGE patterns generated by the petting zoo animals with the national database, no additional human cases were found nor isolates of other origin with identical PFGE patterns.

DISCUSSION

The epidemiological evidence linking the HUS case with a visit to a petting zoo 1 week before the onset of illness, was strengthened by microbiological data showing that strains of STEC O157 isolated from goats and sheep housed on the petting zoo were indistinguishable from the human isolate. Direct animal to human contact or contact with animal manure was the most likely cause of the infection. This is the first time a source of STEC O157 infection has been traced in the Netherlands. Upon subsequent testing of other, randomly selected petting zoos we found STEC O157 on 2 out of 11 (10 petting zoos and 1 deer park). Moreover, during the study there was one more case of STEC O157 infection possibly linked with a farm visit. However, although the petting zoo associated with the case tested positive for STEC O157, the epidemiological link could not be confirmed because the human isolate was not available for subtyping.

Petting zoo visits are popular leisure activities and also have become an important feature of education for young children. In the Netherlands, there are about 500 petting zoos with a total number of about 15–20 million visits annually, mainly in family groups but also in prearranged school parties. Such visits are highly beneficial to children in helping them to learn about aspects of animal husbandry and farm produce. Close contact with the animals is often encouraged, such as petting and feeding animals. However, the above case highlights the risk, especially to the main group of visitors, young children, of acquiring severe

zoonotic infections during visits to petting zoos. Cattle and other ruminants, such as sheep and goats, are important natural reservoirs of STEC O157. Animals carrying STEC O157 usually do not show clinical symptoms and shedding appears to be intermittent and transient [15]. Colonization of cattle with STEC O157 is typically of 2 months or less in duration [16]. Furthermore, shedding appears to be seasonal [5, 15]. Excretion rates peak in the summer and early autumn and are lowest during the winter. The risk of visitors of petting zoos becoming infected with STEC O157 from the livestock or the farm environment appears to be small, given the relatively small number of human cases each year in proportion to the large number of visitors. In the Netherlands, the annual incidence of laboratory-confirmed cases of STEC O157 infection through the enhanced surveillance system is 0·25 per 100000 inhabitants [17]. From the limited data obtained in this study, it also appears that STEC O157 is prevalent in quite a number of petting zoos (in total, 4 of 13 petting zoos (including the deer park) tested positive). In recent years, several other papers have been published about STEC O157 infection among visitors to open farms [18–24]. It has been suggested that sporadic cases may be more closely linked to direct or indirect zoonotic contact with farm livestock than previously suspected [25–30]. Based on the epidemiological data discussed above, direct contact with animals or their manure may also in the Netherlands play a more important role in transmission of STEC O157 than assumed so far. It might even be more important than foodborne transmission. Moreover, contact with livestock animals may result in other human infections, such as campylobacteriosis, salmonellosis, cryptosporidiosis, giardiasis, leptospirosis, ovine *Chlamydia psittaci* infections, Q fever, orf and ringworm. The causative organisms may be present in the animal's milk, urine and faeces (both droppings and on the hide of the animals) and elsewhere in the farm environment.

The findings of the present study engendered discussion about which structural preventive measures to take to reduce the risk of human infection with STEC O157 by visitors to petting zoos. The natural source of infection of farm animals is unknown. Therefore, and given the intermittent pattern of shedding of STEC O157 by individual animals, the capacity of STEC O157 to persist and multiplicate in the farm environment (animal faeces, straw, soil, water) [31] and their natural occurrence in several wild animal species from which interspecies transmission to domestic animals may occur [32], preventing the introduction of the infection, routine testing of brought-in replacement animals, culling infected animals, and closing infected petting zoos, all do not appear to be feasible or effective control measures. Consistent with this, Pritchard et al. [22] found no obvious value in pre-entry bacteriological testing of animals during a longitudinal study on a farm open to the public. Calves apparently not excreting STEC O157 on arrival, later started to excrete STEC O157 of a different strain type than the ones isolated previously on the farm. It is also important to realize that petting zoos not found positive on a first visit may be found positive on a second visit. Longitudinal studies on dairy farms have shown that the STEC O157 status of a farm cannot be ascertained from a single visit, testing a small number of animals. Several studies have been reported on the effects of dietary changes, antimicrobial agents, probiotics, competitive exclusion treatment and immunization on the proliferation and faecal shedding of STEC O157 by animals [33]. However, these studies did not yield unequivocal conclusions. Although STEC O157 strains are excreted intermittently and transiently, it is important to isolate animals known to be infected and those sharing the same paddock or pen away from visitors. In the present study, contact with visitors was allowed again after two successive negative test results, 2–4 weeks apart. Although it is not possible to take specific structural preventive actions at present, the creation of a safe farm environment by the owner and the observance of simple hygienic procedures by visitors themselves are probably the most important preventive measures [34, 35]. Owners should provide good standards of hygiene, adequate toilet and handwashing facilities, separate eating areas from animal contact areas, instructions in simple hygiene measures to visitors, and close supervision of visitors, particularly children. In consultation with the Dutch Foundation for Petting Zoos, guidance for owners and employees of petting zoos has been written with general advice on pathogens likely to be present in the farm environment and steps to be taken to minimize the risk of human infection. Visitors are often not conscious of, or disparage the possible risk of acquiring zoonotic infections through contact with farm animals. Therefore, more attention will need to be paid to informing the public about farm visits and zoonoses, about the risks and their responsibilities. Controlling the risk of STEC O157 infection will also minimize the risks from most other pathogens

commonly present in animals and transmissible to humans by hand to mouth.

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