ORIGINAL ARTICLE

Severe Outbreak of Sorbitol-Fermenting *Escherichia coli* O157 via Unpasteurized Milk and Farm Visits, Finland 2012

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Impacts

- An atypical, sorbitol-fermenting strain of *Escherichia coli* O157 (SF O157) caused an outbreak that involved a high proportion of infected children with severe symptoms, namely haemolytic-uraemic syndrome.
- The source of the outbreak was traced back to a recreational farm selling unpasteurized milk, and microbiological confirmation was obtained, as opposed to the majority of previous reports on SF O157 infections.
- Unpasteurized milk can serve as a vehicle and cattle as a reservoir, for SF O157 causing severe illness in humans.

Keywords:
Shiga toxin-producing *Escherichia coli*; O157; sorbitol-fermenting; outbreak; cattle; milk

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Summary

Shiga toxin-producing, sorbitol-fermenting *Escherichia coli* O157 (SF O157) has emerged as a cause of severe human illness. Despite frequent human findings, its transmission routes and reservoirs remain largely unknown. Foodborne transmission and reservoir in cattle have been suspected, but with limited supporting evidence. This study describes the outbreak of SF O157 that occurred in Finland in 2012. The outbreak originated from a recreational farm selling unpasteurized milk, as revealed by epidemiologic and microbiological investigations, and involved six hospitalized children and two asymptomatic adults with culture-confirmed infection. An identical strain of SF O157 was isolated from patients, cattle and the farm environment, and epidemiologic analysis suggested unpasteurized milk as the vehicle of transmission. This study reports the first milkborne outbreak of SF O157, provides supporting evidence of cattle as a reservoir and highlights the health risks related to the consumption of unpasteurized milk.

Introduction

Shiga toxin-producing *Escherichia coli* (STEC) O157 causes human illness with symptoms such as diarrhoea, haemorrhagic colitis and haemolytic-uraemic syndrome (HUS) (Tarr et al., 2005). The microbiological identification of STEC O157 has traditionally relied on its inability to ferment sorbitol. However, sorbitol-fermenting *E. coli* O157 (SF O157) has emerged as a notable cause of outbreaks and sporadic illnesses in Europe, since first identified in Germany in 1988 (Karch and Bielaszewska, 2001; Editorial team, 2006; Alpers et al., 2009; Orth et al., 2009; King et al., 2014). Illnesses caused by SF O157 have been associated with more severe outcomes, including higher incidence of HUS and mortality (Alpers et al., 2009; Nielsen et al., 2011).

Despite identified outbreaks of SF O157, the transmission routes and reservoirs of this pathogen remain largely unknown. As commonly recognized for non-SF O157, foodborne transmission and reservoir in cattle have also been suspected for SF O157, albeit little supporting evidence exists so far (Bielaszewska et al., 2000; Orth et al., 2006; King et al., 2014). This study presents evidence on the cattle reservoir

and foodborne transmission of SF O157 via unpasteurized milk from an outbreak in Finland in 2012.

Materials and Methods

Identification of the outbreak and its source

On 19 June 2012, the first suspected *E. coli* infection was reported to the environmental health officials at the city of Turku. The patient, a 4-year-old child with HUS, had consumed unpasteurized milk produced on a farm in Turku. The infection was confirmed by a positive Shiga toxin result (Premier® EHEC, Meridian Bioscience, London, UK) at the Turku University Hospital next day. On 21 June, another suspected infection was associated with the consumption of milk from this farm and an outbreak notification was issued to the governmental officials at the National Institute for Health and Welfare (THL) and Finnish Food Safety Authority (Evira).

The suspected farm was a historical tourist sight with 1950s methods in raising cattle and sheep. It had been frequently offering theme visits especially for families and organizing events. The animals were pastured close to pedestrian routes all summer before the outbreak, allowing visitors to touch the animals. Unpasteurized cow's milk had been sold to visitors for several years and possibly served at two events in June 2012. The number of farm visitors or the milk purchasing customers could not be confirmed precisely. To confirm the source of the outbreak and plan control measures, the farm was sampled and inspected, and an online questionnaire was launched for any visitors in June.

Microbiology of human samples

Human samples were examined for STEC by the Turku University Hospital, and the isolates were further characterized at THL. Detection of the virulence genes *stx*1, *stx*2, *eae*, *ehxA* and *saa*, determination of the O:H serotype and sorbitol fermentation and phage typing were performed as described previously (Khakhria et al., 1990; Keskimäki et al., 1998; Paton and Paton, 2002; Eklund et al., 2006). The H genoserotypes of non-motile isolates were determined by real-time PCR (Perelle et al., 2004). Haemolysis was examined on tryptose agar containing 5% of washed sheep blood and 10 mM of Ca_2Cl (Beutin et al., 1989). Antimicrobial susceptibility was tested by using a disc diffusion method according to the Clinical Laboratory Standards Institute (CLSI) for streptomycin, sulphonamide and tetracycline or according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) for ampicillin, chloramphenicol, trimethoprim, ciprofloxacin, gentamicin, nalidixic acid, cefotaxime, mesillanam (amdinocillin) and imipenem. The isolates were genotyped by pulsed-field gel electrophoresis (PFGE) with *Xba*I digestion according to the PulseNet protocol (http://www.cdc.gov/pulsenet/PDF/ecoli-shigella-salmonella-pfge-protocol-508c.pdf).

A representative isolate was subjected to whole-genome sequencing to further examine the presence of virulence genes that distinguish SF O157 from non-SF O157 strains. Genomic DNA libraries were prepared by using Nextera XT kit (Illumina, San Diego, CA, USA) and ran on MiSeq (Illumina) sequencer with 150-bp paired-end reads. FASTQ sequences were submitted to the European Nucleotide Archive (http://www.ebi.ac.uk/ena) under run accession no. ERR1662961.

Reads were mapped against a set of reference genes by using ReMatCh (https://github.com/bfrgoncalves/ReMatCh/tree/course_version) with default settings. Sequences for the reference genes *etpD*, *katP*, *espP* and *terZABCDEF* were extracted from *E. coli* O157:H7 strain Sakai (GenBank accession no. AB011549.2 and NC_002695.1), for the gene *sfpA* from *E. coli* O157:H– plasmid pSFO157 (NC_009602.1) and for the genes *cdtV-ABC* from *Enterobacteria* phage fiAA91-ss (NC_022750.1). The genes belonging to *ter* and *cdt-V* clusters were tested individually. *Stx2* subtype was derived from a reference set consisting of *stx2a* (X07865.1), *stx2b* (X65949.1), *stx2c* (AB071845.1), *stx2d* (AY095209.1), *stx2e* (AJ249351.2), *stx2f* (AB472687.1) and *stx2g* (AY286000.1) (Ashton et al., 2015). All the reference genes were complemented with a 100-bp sequence both upstream and downstream to avoid coverage problems in mapping.

Farm samplings and inspections

The farm was sampled and inspected by the city officials twice: during the outbreak (on 25 June, sampling A) and 3 months later (on 18 September, sampling B). Both times, samples were taken from the livestock, environment and milk (Table 1). The livestock consisted of 15 milking cows, 14 juvenile cattle and 14 sheep. No water samples were taken because of minor contamination risk: drinking water was sourced from a communal supply with regular monitoring, and animals were fenced off from natural waters. Between the samplings, the farm was inspected four times to assess the risk points, and to plan and monitor control measures, aimed at both reducing on-farm infection pressure and preventing transmissions to humans. None of the 12 farm workers reported diarrhoea or other symptoms, but for precaution, six of them were sampled between late July and late August, 2012.

Analysis of farm samples for STEC

Faecal (10 g) and environmental samples were examined for SF O157 by ISO 16654:2001 with modifications and milk samples (2–10 subsamples of 25 mL) by a draft

Table 1. Culture-positive farm samples

Sampling	Sampling A 25 June, during the outbreak		Sampling B 18 September, 3 months later	
Sampling target	No. samples	No. (%) positive samples	No. samples	No. (%) positive samples
Shiga toxin-producing, sorbitol-fermenting *Escherichia coli* O157 (outbreak strain)				
Cattle[a]	16	2 (13)	13	0
Sheep[b]	1	0	6	0[c]
Environment[d]	23	7 (30)	33	0
Milk[e]	8	0[f]	8	0
Campylobacter jejuni				
Cattle[a]	15	4 (27)	12	3 (25)
Sheep[b]	1	0	4	1 (25)
Environment[d]	0	–	1	0
Milk[e]	8	0	8	0
Salmonella[g]	6	0	0	–

[a]Individual faecal samples from rectum.
[b]Composite faecal samples from pastures.
[c]2 (33%) samples positive for Shiga toxin-producing *E. coli* O146:H$^-$.
[d]Swab samples from surfaces in the milk room and from routes, feeding tables and drinking troughs in the barn and outdoors.
[e]Samples from bulk tank milk; sampling A: seven samples collected and refrigerated from 20 through 25 June and 1 sample milked and frozen on 6 May; sampling B: samples collected and refrigerated from 13 through 18 September.
[f]*E. coli* cultures of 4 (50%) samples positive for *stx2* and *eae,* including one culture positive for *rfbE* (O157).
[g]Composite faecal samples from cattle and sheep, three environmental swabs and bulk tank milk.

version of ISO/TS 13136:2012. Faecal samples were enriched for 6 h and environmental samples for both 6 h and 18–24 h. Milk samples were enriched in modified tryptone soya broth supplemented with 12 mg/L acriflavine or in buffered peptone water. Enriched samples were subjected to immunomagnetic separation (Dynabeads® anti-*E. coli* O157, Life Technologies, Oslo, Norway), followed by plating onto selective agars: cefixime-tellurite sorbitol MacConkey agar with 5-bromo-4-chloro-3-indoxyl-β-D-glucuronide (CT-SMAC-BCIG; Harlequin, Lab M, Lancashire, UK) and cefixime-tellurite sorbitol MacConkey agar (CT-SMAC; Difco; Becton, Dickinson and Company, Le Pont de Claix, France) or CHROMagar STEC (CHROMagar, Paris, France). Both CT-SMAC-BCIG and CT-SMAC contained 2.5 mg/L tellurite and 0.05 mg/L cefixime. All the plates were screened for typical colonies that were sorbitol and β-glucuronidase positive.

Typical colonies were biochemically confirmed as *E. coli* and tested for β-glucuronidase activity with 4-methylumbelliferyl-β-D-glucuronide (Okrend, 1990), haemolysis on STEC heart infusion washed blood agar with mitomycin-C (Lin et al., 2012) and O157 antigen agglutination (*E. coli* O157 Latex Test Kit; Oxoid, Thermo Fisher Scientific, Basingstoke, UK). Suspected O157 isolates were examined for the presence of *stx1*, *stx2*, *eae*, *ehxA*, *rfbE* (O157) and *sfpA* (Brunder et al., 2001; Paton and Paton, 2002; ISO/TS 13136:2012). *Stx* genes were subtyped according to the European Union Reference Laboratory for *E. coli* (http://www.iss.it/binary/vtec/cont/EU_RL_VTEC_Method_06_Rev_1.pdf). An isolate negative for *rfbE* (O157) was examined for the O:H serotype and *stx* subtype at the World Health Organization Collaborating Centre for Reference and Research on *Escherichia* and *Klebsiella* (Copenhagen, Denmark) by serologic and PCR methods, respectively. The isolates were genotyped by PFGE, and a representative isolate was phage typed and serotyped as described for the human isolates.

Analysis of farm samples for *Campylobacter* and *Salmonella*

Analyses for *Campylobacter* and *Salmonella* were performed to further assess risks on the farm. Faecal (10 g), environmental and milk samples (1–10 subsamples of 25 mL) were examined for *Campylobacter* by ISO 10272-1:2006 with the exception of enrichment at 41.5°C for 20–24 h. *Salmonella* was examined from faecal and environmental samples by ISO 6579:2002/Amd 1:2007 and from milk by NMKL 71:1999 (Nordic Committee on Food Analysis) at Eurofins Scientific (Raisio, Finland).

Questionnaire study

A case was defined as laboratory-confirmed STEC infection or diarrhoea or blood in faeces between 31 May and 11 July in a person who visited the farm in June 2012. Questionnaire data were collected with an online form, publicly available via the city website from 29 June through 7 July. Questions covered the consumption of unpasteurized milk and food, contacts with cattle and sheep, farm areas visited and visit dates. The exposed were compared with the unexposed by calculating attack rates with 95% CI and Fisher's exact *P* values (Table 2). Data were analysed by using the Stata Data Analysis and Statistical Software version 10.0 (StataCorp, College Station, TX, USA).

Results

Microbiological analysis

Altogether, eight culture-confirmed STEC infections were identified, including five cases and three secondary infections. Secondary infections were acquired without visiting the farm and were diagnosed in persons whose family member had consumed the milk. Of the infected persons,

Table 2. Exposures for illness caused by sorbitol-fermenting *Escherichia coli* O157 in farm visitors, Finland 2012

	Exposed	Unexposed		Univariate analysis	
Exposure	Cases/non-cases	Cases/non-cases	Relative risk	95% CI	*P* value
Consumption of unpasteurized milk	6/16	5/110	**6.27**	2.10–18.76	**0.0003**
Animal contact	6/68	5/62	1.09	0.35–3.40	0.89
Visit to pastures	3/51	8/81	0.62	0.17–2.23	0.46
Visit to the barn	1/19	10/109	0.56	0.08–4.40	0.60
Food consumption	5/72	6/58	0.69	0.22–2.16	0.53

Boldface highlights significant risk at 95% confidence level.

two were asymptomatic adults with secondary infection and six were symptomatic children, 1–7 years of age. All the children were hospitalized, four (67%) of them with HUS. Samples from the asymptomatic farm workers tested negative for STEC. All the human isolates represented indistinguishable characteristics: serotype O157:H7 (non-motile), sorbitol-fermenting, β-glucuronidase positive, weakly enterohaemolytic, phage type 88 and pulsotype 1.192. The isolates harboured the genes *stx2*, *eae* and *ehxA* and showed susceptibility to all of the tested antimicrobial agents, 12 in total. Additionally, whole-genome sequencing of a representative human isolate revealed presence of the genes *sfpA*, *etpD* and *cdtV-ABC* and absence of the genes *katP*, *espP* and *terZABCDEF*. *Stx2* subtyped as *stx2a*.

In sampling A, nine (19%) farm samples tested culture positive for a SF O157 strain, indistinguishable from the human isolates (Table 1). This outbreak strain was isolated from cattle faeces and the environment, both in the barn and outside: milk room floor, feeding table, drinking troughs and cattle shelter. All the isolates were recovered from CT-SMAC-BCIG plates containing 2.5 mg/L tellurite. Further, SF O157 was most frequently recovered from environmental samples after enrichment for 18–24 h; only one sample tested positive after 6 h. *E. coli* cultures from milk simultaneously generated positive PCR signals for *stx2*, *eae* and *rfbE* (O157), but attempts failed in obtaining the outbreak strain as pure culture from milk.

In sampling B, samples from cattle, sheep, milk and the farm environment tested negative for SF O157. However, two faecal samples from sheep (flocks untested in sampling A) tested culture positive for STEC O146:H$^-$. These sheep isolates harboured the genes *stx1c*, *stx2b* and *ehxA* and represented pulsotype 1.146.

In addition to STEC, *Campylobacter jejuni* was detected from four (25%) faecal samples of cattle in sampling A and from four (25%) in sampling B. *C. jejuni* was shed by the same animals in both samplings, and one cow simultaneously shed both *C. jejuni* and SF O157. No *Campylobacter* was detected in milk. All the samples analysed for *Salmonella* tested negative.

Questionnaire study

The questionnaire was answered by 146 persons, but the response rate remained undefined because of the unknown number of farm visitors. Of the respondents, 11 (8%) met the case definition, with symptom onset between 7 June and 1 July 2012. Of these case patients, five had laboratory-confirmed STEC infection and seven (64%) were females. The mean age of case patients was 7 years (range 1–41 years). In the univariate analysis, persons who had consumed unpasteurized milk were six times more likely to become ill than non-consumers (relative risk 6.3, 95% CI 2.1–18.8, $P < 0.05$; Table 2). Besides visiting the farm, all the laboratory-confirmed case patients had consumed unpasteurized milk produced there, contrary to five persons meeting the clinical case definition alone. One of the latter had a chronic, diarrhoeic disease and another one exceeded the typical incubation period for STEC infection. Three case patients with non-laboratory-confirmed infection had possibly touched animals during their visit.

Farm inspections and control measures

Immediately after the first report of suspected infection, the sale of unpasteurized milk was prohibited from the farm. Contacts were restricted between farm animals and visitors. The public was informed about the suspected outbreak by signboards on the farm premises and through media.

The farm inspections revealed several deficiencies in milking hygiene, animal husbandry, farm operations and infrastructure, including poor farm hygiene, insufficient washing of udder cloths and excessive animal density. The farm infrastructure lacked the legislative requirements for separating the milk room from other operations; for example, dirty equipment was washed next to the milk tank. Milk was manually milked into a pitcher on the stall floor and then poured into the tank without cleaning the pitcher surface, thus risking the bulk tank milk for manure contamination.

Since spring 2012, large amounts of cattle manure had temporarily been stored in a shelter that animals could freely access from the barn. Simultaneously, the udder health had deteriorated with many cows suffering from mastitis. The quality of the bulk tank milk, however, had usually appeared good based on somatic cell counts and total bacterial counts of <250 000/mL and <50 000 cfu/mL, respectively.

The control measures included cleaning the barn and routes from manure, and continual disinfection of the routes and contaminated areas with calcium oxide. The drinking and feeding troughs in the barn and on pastures were disinfected daily with 1% solution of Virkon® S (DuPont, Suffolk, UK) or powder disinfectant (e.g. Stalosan® F, Stormøllen, Tureby, Denmark). The milk room was replaced, and hygienic practices in milking and animal husbandry were emphasized by guidance.

Discussion

This report presents one of the first reported outbreaks of SF O157 with a microbiologically confirmed source in cattle. Further, the outbreak reported herein represents, to date, Finland's largest STEC outbreak with severe sequelae that was traced back to animal or food source. As suggested by the epidemiologic and microbiological investigations, the outbreak was caused by a SF O157 strain that probably was transmitted via the consumption of unpasteurized cow's milk from the farm in Turku. The outbreak involved five culture-confirmed and six non-laboratory-confirmed case patients, and three persons with secondary, culture-confirmed infection. Altogether, six children with culture-confirmed infection were hospitalized, four (67%) of them with HUS. No infections were reported after restricting farm visits and animal contacts and prohibiting sale of unpasteurized milk from the farm. The farm discontinued dairy farming the following year.

Based on the questionnaire study, the consumption of unpasteurized milk was associated with the illness. The suspicion of unpasteurized milk as the outbreak vehicle was released countrywide before launching the questionnaire, which may have influenced the respondents. *E. coli* cultures from the milk samples tested positive in PCR, suggesting presence of the outbreak strain in milk, despite failure of obtaining it as a pure isolate for confirmation by PFGE. Isolation of the outbreak strain was possibly hindered by the presence of other *E. coli* that alone indicated manure contamination of the milk. Isolates indistinguishable from the patient isolates were recovered from cattle faeces and the farm environment, confirming the farm as the source of the outbreak. Infections that remained unexplained by milk consumption were possibly acquired via direct contact with cattle or the farm environment.

Non-SF O157 admittedly transmits via unpasteurized milk and contact with cattle (Hussein and Sakuma, 2005). However, little is known about the epidemiology, transmission routes and reservoirs of SF O157. Few reports exist on human transmissions via direct contact with cattle and the consumption of ground beef (Bielaszewska et al., 2000; Orth et al., 2006; King et al., 2014). Furthermore, transmissions via unpasteurized cow's milk and raw beef-containing sausage have been suspected, but without microbiological confirmation (Ammon et al., 1999; Allerberger et al., 2001). Allerberger et al. (2001) reported a sporadic infection preceded by the consumption of unpasteurized milk. This report provides, in contrast to theirs, epidemiologic evidence on the transmission via unpasteurized milk and reinforces microbiological evidence on the cattle reservoir. Other sources of SF O157 infection include person-to-person contact and a direct contact with a pony (Karch and Bielaszewska, 2001; Orth et al., 2006). In addition, the environment, apple juice and quark cheese have been suspected without microbiological confirmation (Alpers et al., 2009; Nielsen et al., 2011).

Thus far, human illnesses caused by SF O157 have mainly been reported in Germany and elsewhere in Europe. Despite frequent human findings, SF O157 has seldom been isolated from cattle or other animals in Germany, thus leading to the proposition of a human reservoir (Karch and Bielaszewska, 2001). However, recent studies have reported isolation of SF O157 from ruminant sources outside Europe: retail beef in Egypt, cattle faeces in Mexico and beef carcasses in Turkey (Sallam et al., 2013; Ayaz et al., 2014; Narváez-Bravo et al., 2015). Although the ability of these isolates to cause human illness remained unknown, and some of them lacked *stx* genes, these studies suggest ruminant reservoir and wider distribution in the world. As suggested by Kossow et al. (2016), *stx*-negative SF O157 strains harbouring the gene encoding for H7 antigen ($fliC_{H7}$) possibly represent STEC that lost their *stx*. The presence of $fliC_{H7}$ was confirmed in the Egyptian, Mexican and Turkish studies. Unexpectedly, the Egyptian study reported dominance of SF O157 harbouring $fliC_{H7}$ among STEC O157 isolates from retail beef.

In Finland, SF O157 caused approximately 40 (annually 0–7) sporadic human illnesses in 1997–2015 (Eklund et al., 2006) (THL, unpublished results). In addition, four illness clusters were recognized in 2005, 2012, 2013–2014 and 2016. Excluding the cluster of 2012 reported herein, the source of these infections remained unknown. In 2013–2014, 21 infections with an identical SF O157 isolate were recognized geographically dispersed in Finland, raising suspicion of a retail food source. According to the Epidemic Intelligence Information System, no infections caused by this strain had been identified elsewhere in Europe or the United States (Jaakola et al., 2015). In summer 2016, SF

O157 infections occurred in three families living close to Turku. These infections were caused by a strain that shared an identical pulsotype with the outbreak strain of 2012. This strain was also isolated from sheep raised by one of the infected families. The infection source is under investigation (THL and Evira, unpublished results).

In addition, the outbreak strain of 2012 shared an identical pulsotype with two sporadic human isolates, found months earlier and geographically dispersed, and close relatedness with other human SF O157 isolates collected in Finland (Eklund et al., 2006; Feng et al., 2007) (data not shown). This finding shows concordance with the evolutionary model proposing genomic conservation of SF O157 ($fliC_{H7}$) strains (Feng et al., 2007). Excluding the aforementioned findings, no isolates of the outbreak type have been isolated from other sources, including cattle, in Finland. Overall, only one Finnish isolate of SF STEC O157, distinct from the outbreak strain and harbouring $fliC_{H7}$, has been found from cattle or animal sources in 1999 (Evira, unpublished results). However, SF O157 has only been monitored projectwise and is probably undiscovered from non-human sources.

SF O157 is commonly underdiagnosed because of a lack of routine detection methods. Detection of O157 has traditionally relied on non-SF phenotype on SMAC agar, thus leaving SF strains undetected unless combined with other laborious screening steps. Furthermore, SMAC plates are commonly supplemented with tellurite. Susceptibility to tellurite and consequent inability to grow on CT-SMAC have been reported for most SF O157 strains (Bielaszewska et al., 2005). Contrary to major findings, moderate ability to grow on CT-SMAC-BCIG with 2.5 mg/L tellurite was observed for the outbreak strain in this study, enabling its detection. However, the strain seemed to lack *ter* gene cluster, suggesting another mechanism for tellurite tolerance. No SF O157 could be recovered from CHROMagar STEC because of poor growth, as concordant with previous results (Hirvonen et al., 2012).

Overall, growth of the outbreak strain appeared slower than that of non-SF O157. Non-SF O157 has typically been recovered from environmental samples after 6 h of enrichment (Evira, unpublished results), whereas the outbreak strain predominantly after 18–24 h, when investigated parallel for both incubation times. Slower growth may have crucially hindered isolation of the outbreak strain from milk. In general, milk contains rich endogenous microbiota like other farm samples but notably fewer target cells for isolation, making detection more challenging.

The outbreak strain displayed many features, reported as predominant for SF O157 (Karch and Bielaszewska, 2001). These included non-motility despite presence of $fliC_{H7}$, β-glucuronidase activity, phage type 88 and presence of the genes *stx2, eae, ehxA*, *sfpA*, *etpD* and *cdtV-ABC*, and absence of the genes *katP*, *espP* and *terZABCDEF*. Distinctively, presence of *ehxA* was coupled with a haemolytic phenotype, previously observed for the minority of SF O157. In addition, SF O157 infections have been reported with higher incidence of HUS among children than non-SF O157 infections (Alpers et al., 2009; Nielsen et al., 2011). Concordantly, this study showed HUS in 67% of the infected children. With non-SF O157 infection, HUS develops in ≈15% of children younger than 10 years (Tarr et al., 2005). However, asymptomatic carriage was also detected in two adults, as previously reported for SF O157 (Orth et al., 2006, 2009; Nielsen et al., 2011).

In addition to SF O157, *C. jejuni* was isolated from faecal samples of cattle and was shed by the same animals in both samplings, indicating persistence. Long-term persistence and shedding of *C. jejuni* have previously been observed in dairy cattle (Hakkinen and Hänninen, 2009). Distinctively, this study demonstrated persistence of *C. jejuni* in cattle despite the control measures aimed at reducing on-farm infection pressure of STEC. No shedding of SF O157 was detected after application of these measures, suggesting their effect against STEC. Therefore, different control approaches may be needed for different enteric pathogens. As stated previously, no effective on-farm control practices exist for *C. jejuni*, as opposed to STEC (Adam and Brülisauer, 2010).

This outbreak highlights the well-recognized risk related to the consumption of unpasteurized milk. As indicated by several outbreaks, unpasteurized milk can be contaminated with a variety of pathogens, shed by cattle in their faeces (Oliver et al., 2009). The risk of pathogen contamination can be reduced by good production hygiene, but not eliminated completely. As Ruusunen et al. (2013) observed, hygiene indicators (*E. coli* and total bacterial counts) failed to indicate the presence of pathogenic bacteria in bulk tank milk. Concordantly, no correlation between the total bacterial counts and risk of illness was observed in this study. Therefore, heating unpasteurized milk is recommended to assure its safe consumption. Along with established pathogens, contamination by emerging variants, such as SF O157, has to be considered when choosing laboratory methods and planning surveillance.

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