Foodborne transmission of sorbitol-fermenting *Escherichia coli* O157:[H7] via ground beef: an outbreak in northern France, 2011

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Abstract

Sorbitol-fermenting *Escherichia coli* O157:[H7] is a particularly virulent clone of *E. coli* O157:H7 associated with a higher incidence of haemolytic uraemic syndrome and a higher case fatality rate. Many fundamental aspects of its epidemiology remain to be elucidated, including its reservoir and transmission routes and vehicles. We describe an outbreak of sorbitol-fermenting *E. coli* O157:[H7] that occurred in France in 2011. Eighteen cases of paediatric haemolytic uraemic syndrome with symptom onset between 6 June and 15 July 2011 were identified among children aged 6 months to 10 years residing in northern France. A strain of sorbitol-fermenting *E. coli* O157:[H7] *stx2a eae* was isolated from ten cases. Epidemiological, microbiological and trace-back investigations identified multiply-contaminated frozen ground beef products bought in a supermarket chain as the outbreak vehicle. Strains with three distinct pulsotypes that were isolated from patients, ground beef preparations recovered from patients' freezers and from stored production samples taken at the production plant were indistinguishable upon molecular comparison. This investigation documents microbiologically confirmed foodborne transmission of sorbitol-fermenting of *E. coli* O157 via beef and could additionally provide evidence of a reservoir in cattle for this pathogen.

Keywords: *E. coli* O157, France, haemolytic uraemic syndrome, outbreak, Shiga toxin-producing *E. coli*
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Introduction

Enterohaemorrhagic *Escherichia coli* (EHEC) O157:H7, also known as pathogenic Shiga toxin-producing *E. coli* (STEC) O157:H7, is an established worldwide cause of diarrhoeal illness and haemolytic uraemic syndrome (HUS). The inability of this EHEC serotype to ferment sorbitol is a phenotypic characteristic key to its microbiological identification.

However, non-motile strains of EHEC O157:[H7] capable of fermenting sorbitol (SF) have also emerged as important human pathogens in continental Europe since their identification in southern Germany in 1988 [1–4]. Although more rarely isolated from human patients than classic EHEC O157:H7, preliminary evidence suggests that SF O157:[H7] infections are associated with a higher HUS incidence and case fatality rate [2–4].

We present here the investigation of a foodborne outbreak of SF EHEC O157:[H7] that was identified on 14 June 2011 following the diagnosis of five paediatric HUS cases between 10 and 13 June in a single administrative district in northern France.

Methods

A confirmed case was defined as a person living in France who presented with bloody diarrhoea or HUS between 1 June and 24 July 2011 and had isolation of an outbreak strain from a stool. An outbreak strain was defined as an SF *E. coli* strain with the same serotype and pulsotype as a strain isolated from the incriminated food, namely O157:[H7] (pulsotypes C or E) and O177:[H25] (pulsotype A). A probable case was a person living in the same administrative district as a confirmed case with bloody diarrhoea or HUS between 1 June and 24 July 2011 and who had a serology positive for *E. coli* O157 without strain isolation.

A trawling questionnaire based on exposure to known EHEC risks exposures in the week before symptom onset was administered to parents of the initial five cases. Consumption of ground beef preparations from a single chain of supermarkets was rapidly identified as the unique risk exposures common to cases and thus a more detailed specific ground-beef questionnaire was developed and employed.

Stool samples or rectal swabs collected from hospitalized patients with HUS were plated on D-sorbitol MacConkey agar (SMAC; Becton Dickinson GmbH, Heidelberg, Germany) and on Drigalski lactose agar (bioMerieux SA, Marcy-l'Etoile, France). An enrichment step was also performed in 10 mL of Trypto-casein-soy broth (Bio-Rad, Marnes-la-Coquette, France) for 4–6 h and 10-μL aliquots were plated on SMAC and on Drigalski. The cultures were incubated aerobically at 36 $\pm$ 2°C for 18–24 h. The overnight cultures were examined for bacterial growth and colony morphology. Sorbitol fermenting colonies were selected from the culture plate and tested in O157 latex reagent (Oxoid, Wesel, Germany). The biochemical properties of the isolated strains were determined by standard methods for identifying *E. coli*, and D-Sorbitol fermentation was examined in the tube test after 18 h by the API 20E system (bioMerieux SA). PCR for virulence genes *stx1*, *stx2*, *eae* and EHEC-*hlyA* was performed on broth and isolated colonies as previously described [5,6].

Serum samples from cases were examined for IgM and IgA antibodies to the lipopolysaccharide of eight major EHEC serogroups by line blot immunoassay as described previously [7].

Food samples underwent two parallel analyses: (i) detection of *E. coli* O157 on 50-g samples according to the NF EN ISO 16654:2001 method [8] using the French Association for Standardisation (AFNOR)-validated alternative method VIDAS® UP *E. coli* O157 including H7 (bioMérieux); (ii) investigation of the presence of STEC strains on 25-g samples according to the XP CEN ISO/TS 13136:2012 method [9].

Serotyping was performed by agglutination, PCR/RFLP, *rfb*-RFLP or *fliC* sequencing as described previously [6,10].

Determination of *stx* and *eae* variants was performed by PCR as described previously [11,12]. We additionally investigated the presence of several characteristics that are considered typical or specific for SF EHEC O157 of the 'German clone' as opposed to classical EHEC O157. Using a representative subset of isolated SF O157 outbreak strains (both outbreak pulsotypes; human strains, strains recovered from ground beef in case homes and those recovered from the production site), we carried out analyses for the presence of the *sfp* cluster, EHEC-*hlyA*, *etpD*, *espP*, *katP*, *iha* and the *ter* cluster by PCR as described previously [1,13–18].

The genetic relatedness of human and food strains was studied by using the Standard PulseNet PFGE protocol for *E. coli* O157 [19].

A trace-back investigation was carried out to identify the source of ground beef contamination. A trace-forward investigation identified the list of contaminated products and their distribution chain.

Results

Eighteen cases (12 confirmed and six probable) with symptom onset between 6 June and 15 July 2011 were identified in six administrative districts in north and north-east France (Figs 1 and 2). Fourteen (77%) cases lived in two neighbouring districts in northern France (Nord, $n = 12$; Pas-de-Calais, $n = 2$). All cases were hospitalized for post-diarrhoeic HUS. The median age was 3 years (range: 6 months–10 years) and the sex ratio was 1.0. No deaths occurred. Two secondary cases (confirmed) occurred in two households following intra-familial transmission from a sibling. No cases of bloody diarrhoea without HUS development were identified.

The patients did not attend a common childcare institution or school and did not know each other. The unique common risk exposure was consumption of ground beef preparations. Fourteen (87%) of the 16 primary cases reported consumption of ground beef products (purchased frozen) in the week before symptom onset (Table 1). Eleven (79%) of them purchased ground beef products in supermarket chain 'X' (beef burger ($n = 8$), onion burger ($n = 1$), hamburger ($n = 1$), beef burger and meat balls ($n = 1$)) and 10 (90%) of these 11 cases had consumed preparations of a single brand 'A'. For one of the secondary cases, a 7-month-old baby, an older brother with non-bloody diarrhoea had consumed an onion burger of brand 'A' that was purchased in the supermarket chain 'X'.

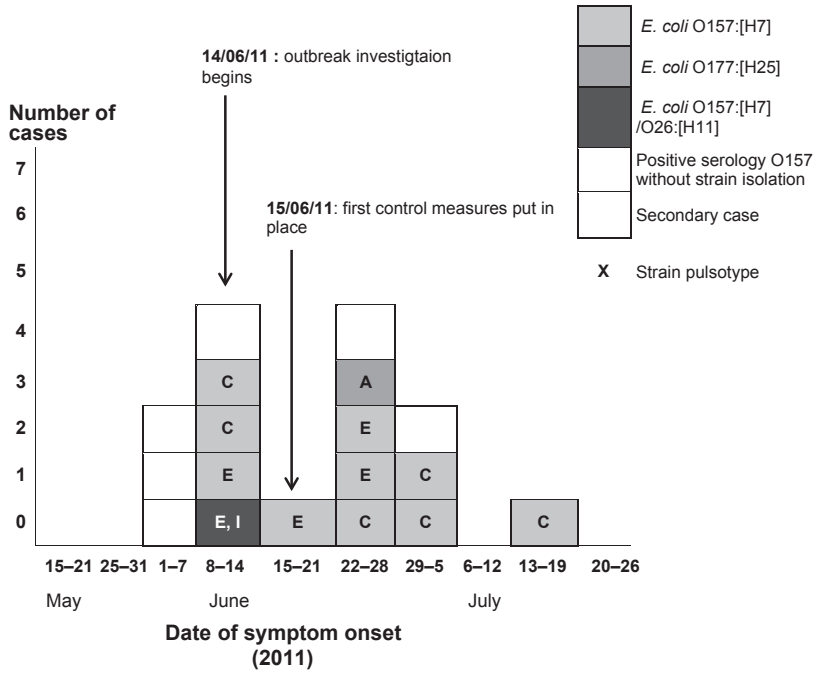


FIG. 1. Number of *E. coli* O157:[H7]/O177:[H25] outbreak cases by date of symptom onset, Shiga toxin-producing *E. coli* serotype and pulsotype of isolated strains, France, 2011 (n = 18).

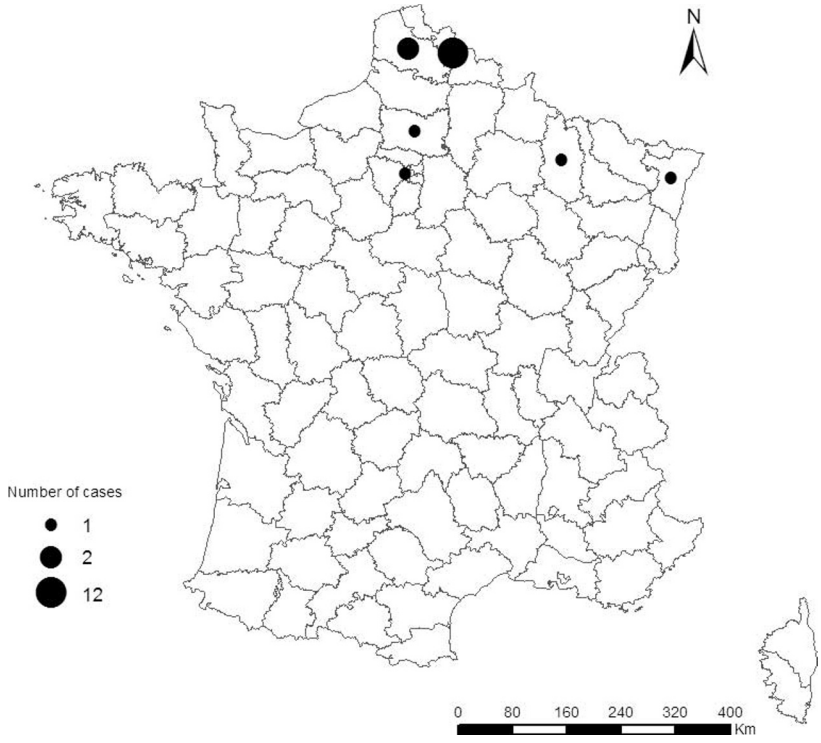


FIG. 2. Number of *E. coli* O157:[H7]/O177:[H25] outbreak cases by administrative district of residence, France, 2011 (n = 18).

A precise supermarket chain 'X' store where the beef preparation was purchased was identified for ten case households. Nine distinct stores were cited, with two families reporting a single store. These nine stores were located in five administrative districts in north (n = 8) and north-east (n = 1) France; seven were located in the two districts in north France where 77% of identified cases resided.

Stool samples of 13 of the 15 cases sampled yielded positive results (no sample was available for three cases): 11 cases with an EHEC strain (10 O157:[H7], one O177:[H25]) and one case

with two attaching and effacing *E. coli* (AEEC) strains (O157:[H7], O26:[H11]; Table 1). The thirteenth case was PCR positive for O157 without strain isolation. All isolated *E. coli* strains fermented sorbitol. The virulence profile *stx2 eae* was present in all 11 isolated EHEC strains. The EHEC-*hlyA* gene was present in nine (eight O157:[H7] and one O177:[H25]) of the EHEC strains and one AEEC strain (O157:[H7]). Six O157:[H7] strains were pulsotype C, five, including the AEEC strain, were pulsotype E, the O26:[H11] strain was pulsotype I and the O177:[H25] strain was pulsotype A (Table 1, Fig. 3). Two patients had evidence of EHEC infection with two distinct serogroups: O157-O26; O157-O177.

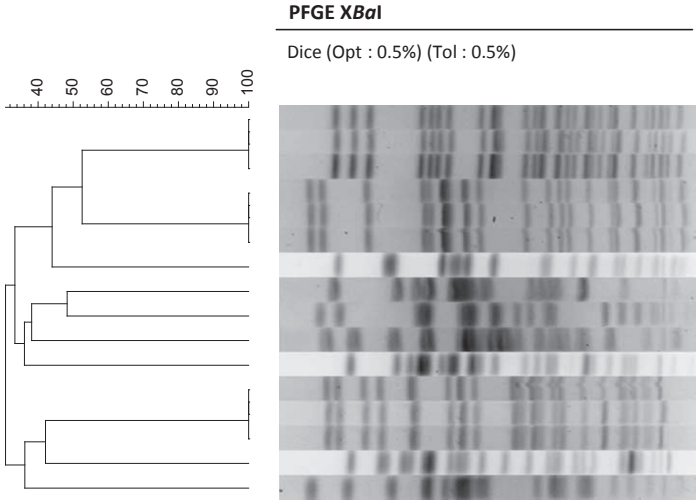
Sixteen cases, including ten confirmed cases, had a serology positive for *E. coli* O157 (Table 1).

Leftover frozen ground beef preparations were recovered from the homes of eight case families, including six families that ate preparations of brand 'A' from supermarket 'X' (brand 'A', beef burgers, $n = 4$, onion burgers, $n = 2$; brands 'B' and 'C', beef burgers, $n = 2$). The packaging was available for recovered beef burgers of brand 'A' from three of the six families; all these beef burgers had been produced by a single producer 'E' on 11 May 2011. All six recovered preparations of brand 'A' tested positive for STEC/AEEC while the beef burgers from two other brands tested negative (Table 1). Isolated strains fermented sorbitol and belonged to five distinct serotypes (O157:[H7], O177:[H25], O26:[H11], O116:H21 and O_{rough}:[H7]) and pulsotypes (A, B, C, E and H).

TABLE 1. Sorbitol-fermenting *Escherichia coli* O157:[H7] outbreak, France, 2011: results of microbiological and serological analysis of cases and of microbiological analysis of ground beef preparations recovered from the homes of cases (when available), combined with epidemiological results. (Cases are ordered by case strain pulsotype)

Case	Case serology result	Case microbiology result[a] (*strain name*)	Case strain pulsotype	Ground beef product consumed; product brand	Supermarket chain of purchase	Isolated food strain(s)[a] (*strain name*)	Food strain pulsotype
1	O157	SF O177:[H25] *stx2 eae* (*32706*)	A	Beef burger; A	X	No remaining ground beef for testing	
2	O157	SF O157:[H7] *stx2 eae* (*32667*)	C	Onion burger; A	X	SF O157:[H7] *stx2 eae* (*767*)	C
3	Negative	SF O157:[H7] *stx2 eae* (*32737*)	C	No reported consumption (secondary case; older brother with non-bloody diarrhoea consumed an onion burger; A)[b]	X	SF O157:[H7] *stx2 eae* (*967*) SF O177:[H25] *stx2 eae* (*966-19*)	C A
4	O157	SF O157:[H7] *stx2 eae* (*32738*)	C	No reported consumption[c]			
5	O157	SF O157:[H7] *stx2 eae* (*32735*)	C	No reported consumption[c] (secondary case)			
6	Not tested	SF O157:[H7] *stx2 eae* (*32850*)	C	No reported consumption			
7	O157	SF O157:[H7] *stx2 eae* (*32617*)	C	Undetermined type of beef product (supermarket X); unknown; beef burger (supermarket Y); B	X, Y	Absence of pathogenic STEC (beef burger from supermarket Y)	
8	O157	SF O157:[H7] *stx2 eae* (*32616*)	E	Beef burger; A	X	SF O157:[H7] *eae* (*481-1*) SF Orough:[H7] *stx2 eae* (*481-16-3*) SF O157:[H7] *stx2 eae* (*481-16-1*) SF O116:[H21] *stx2* (*481-15-1*)	E C C B
9	O157	SF O157:[H7] *stx2 eae* (*32739*)	E	Beef burger; A	X	SF O157:[H7] *eae* (*872*)	E
10	O157	SF O157:[H7] *stx2 eae* (*32736*)	E	Beef burger and meat balls; unknown and A	X	No remaining ground beef for testing	
11	O157	SF O157:[H7] *stx2 eae* (*32715*)	E	Beef burger; A	X	No remaining ground beef for testing	
12	O157	SF O157:[H7] *eae* (*32665*) SF O26:[H11] *eae* (*32633*)	E (O157) I (O26)	Beef burger; A	X	SF O157:[H7] *eae* (*460-13-CMC*)	E
13	O157	O157 *stx2 eae* (PCR result without strain isolation)	/	Beef burger; A	X	No remaining ground beef for testing	
14	O157	Negative	/	Beef burger; A	X	SF O157:[H7] *eae* (*463-3*) SF O177:[H25] *stx2 eae* (*469-15-7*) SF O26:[H11]) *stx1 eae* (*476-21*)	E A H
15	O157	Not tested	/	Beef burger; C	Z	Absence of pathogenic STEC	
16	O157	Negative	/	Beef burger; A	X	No remaining ground beef for testing	
17	O157	Not tested	/	Hamburger; unknown	X	No remaining ground beef for testing	
18	O157	Not tested	/	Meat balls and steak tartar; unknown	V and W	No remaining ground beef for testing	

[a]SF, sorbitol-fermenting strain; [HX], non-motile strain; O157 has been determined either by agglutination (clinical isolates) or PCR (food isolates); O26 has been determined either by *rfb*-RFLP (clinical isolates) or PCR (food isolates); O177 and O116 have been determined by *rfb*-RFLP; H alleles have been determined by PCR (H7 and H11) or by *fliC* sequencing (H21, H25).
[b]Case no. 3 is a secondary case due to human transmission; the analysed onion burgers came from the box of burgers from which his older, symptomatic brother had eaten.
[c]Two cases from the same family.



Isolate	Serotype (a)	PFGE type	Source
32735	O157:[H7]	C	Human case n°5 (b)
632	O157:[H7]	C	Industrial meat (c)
967	O157:[H7]	C	Consumer meat (Human case n°3) (d)
481–1	O157:[H7]	E	Consumer meat (Human case n°8) (e)
32616	O157:[H7]	E	Human case n°8 (f)
549	O157:[H7]	E	Industrial meat (g)
481–15–1	O116:[H21]	B	Consumer meat (Human case n°8)
955	Onew:H28	N	Industrial meat
1062	O103:H2	L	Industrial meat
954	O103:H2	M	Industrial meat
32633	O26:[H11]	I	Human case n°12
966–19	O177:[H25]	A	Consumer meat (Human case n°3) (h)
958	O177:[H25]	A	Industrial meat (i)
32706	O177:[H25]	A	Human case n°1
476–21	O26:[H11]	H	Consumer meat (Human case n°14)
863	O22:H8	K	Industrial meat

FIG. 3. Sorbitol-fermenting *Escherichia coli* O157:[H7] outbreak, France, 2011: *XBa*I pulsed-field gel electrophoresis patterns, serotype and source of the *E. coli* strains isolated from human cases, consumer meat and industrial meat. The dendrogram was generated using the band-based Dice similarity coefficient with 0.5% band position tolerance and the unweighted pair group method with arithmetic mean clustering. (a) [HX]: non-motile strain; O157 has been determined either by agglutination (clinical isolates) or PCR (food isolates); O26 has been determined either by *rfb*-RFLP (clinical isolates) or PCR (food isolates); O103 has been determined by PCR; O22, O26, O177 and O116 have been determined by *rfb*-RFLP; H alleles have been determined by PCR (H2, H7, H8 and H11) or by *fliC* sequencing (H21,H25, and H28). (b–d) PFGE type C is also shared by isolates from (*data not shown*): (b) human cases n°2, 3, 4, 6 and 7 (isolates n°32667, 32737, 32738, 32850 and 32617, respectively); (c) industrial meat (isolates n°540; 593; 609; 620; 668; 52-33n°34 and 1061-A); (d) consumer meat of human cases n°2 and 8 (isolates n°767, 481-16-3 and 481-16-1, respectively). (e–g) PFGE type E is also shared by isolates from (*data not shown*): (e) consumer meat of human cases n°9, 12 and 14 (isolates n°872, 460-13-CMC and 463-3, respectively); (f) human cases n°9, 10, 11 and 12 (isolates n°32739, 32736, 32715 and 32665, respectively); (g) industrial meat (isolate n°603). (h and i) PFGE type A is also shared by isolates from (*data not shown*): (h) consumer meat of human case n°14 (isolate n°469-15-7); (i) industrial meat (isolates n°554, 851, 958 and 1061-B).

All isolated SF O157 strains possessed the *stx2a* and *eae-γ* variants (Table 2). The results of complementary analyses that examined the presence of characteristics considered specific for SF EHEC O157 were coherent with SF O157 of the 'German clone'(Table 2).

Indistinguishable pulsotypes C, E and A were identified for EHEC strains isolated from human and food samples for the five families where comparison was possible (Table 1, Fig. 3).

Some frozen ground beef preparations recovered from producer 'E' and produced between 6 and 23 May 2011 from

TABLE 2. Sorbitol-fermenting *E. coli* O157:[H7] outbreak, France, 2011: results of complementary microbiological analyses investigating the presence of molecular characteristics considered typical or specific for SF *E. coli* O157 of the 'German clone' (the table is ordered by strain pulsotype)

Strain origin (*strain name*)	Strain pulsotype	*eae* variant	*stx2* variant	EHEC-*hlyA*	*sfpA*	*etpD*	*espP*	*katP*	*iha*	*ter* cluster genes[a]
Human case no. 8 (*32616*)	E	γ	*stx2a*	+	+	+	–	–	–	–
Consumer meat (human case no. 8) (*481-1*)	E	γ	–[b]	+	+	+	–	–	–	–
Industrial meat (*549*)	E	γ	–[b]	+	+	+	–	–	–	–
Human case no. 5 (*32735*)	C	γ	*stx2a*	+	+	+	–	–	–	–
Consumer meat (human case no. 3) (*967*)	C	γ	*stx2a*	+	+	+	–	–	–	–
Industrial meat (*632*)	C	γ	*stx2a*	+	+	+	–	–	–	–
Control strain : SF EHEC O157:H7 of the 'German clone' (*CB 2755*)[c]	Different from E and C	γ	*stx2a*	+	+	+	–	–	–	–
Control strain: classical non-SF O157:H7 (*1316*)[d]	Different from E and C	γ	*stx2c*	+	–	+	+	+	+	+

[a]The *terA*, *terB*, *terC*, *terD*, *terE*, *terF*, *terZ*, *terW* and *terY* genes have been tested.
[b]*stx*-negative strain.
[c]Strain provided by Dr L. Beutin of the National Reference Laboratory for *Escherichia coli*, Federal Institute for Risk Assessment (BfR), Berlin, Germany.
[d]Strain isolated from food (industrial meat) in France in 2011.

the same batches of raw beef tested positive for STEC/AEEC strains (O157:[H7], O177:[H25], but also O103:[H2]; Onon-typable:H28 and O22:H8). Strains isolated from food fermented sorbitol and belonged to seven distinct pulsotypes (A, C, E, K, L, M and N; Fig. 3). Strains with five distinct pulsotypes were recovered from ground beef preparations that were produced on 11 May.

Strains with pulsotypes A, C and E that were isolated from cases, ground beef preparations recovered from cases' freezers and from stored production samples taken at production plant 'E' were indistinguishable by PFGE (Fig. 3).

The implicated batches of raw beef consisted of 60 tons of beef imported from three German suppliers. All STEC-contaminated preparations were made with beef from one supplier, either exclusively or combined with raw material from the two other suppliers. Several brands, including brand 'A', were supplied with ground beef preparations produced between 5 and 23 May 2011 from the implicated batches of raw beef. On 11 May, 13.6 tons of beef burgers had been produced for brand 'A'.

Products fabricated by the producer 'E' from the incriminated batches of beef and distributed for sale were sold from early May 2011 in several supermarket chains. Seventy-three per cent of these products had been distributed to supermarket chain 'X' (brand 'A'). Eighty-six per cent of the products that were made on 11 May with the implicated batches of raw beef and distributed for sale had been made for supermarket chain 'X'. The traceability system used by chain 'X' did not enable identification of the exact supermarkets that received products made from these batches of raw beef nor the exact quantities delivered. However, analysis of delivery platform data enabled identification of supermarket 'X' stores likely to have received these products for sale. Stores were identified in 57 administrative districts across France but with a majority in the northern half of the country. Retrospective analysis of the records of producer 'E' suggests that 83% of the beef burgers of brand 'A' produced on 11 May were delivered to a single distribution platform that served supermarket 'X' stores in the two districts in northern France where 77% of cases resided, in addition to a single store in a third district where no cases were identified.

Two successive national recalls of contaminated products were issued on 15 June and 2 July 2011 following the evolution of the epidemiological investigations. France issued multiple Europe-wide alert messages related to this outbreak via the Early Warning Response System and the Rapid Alert System for Food and Feed of the European Commission and via the Epidemic Intelligence Information System of the European Centre for Disease Prevention and Control starting on 16 June. No countries reported the occurrence of cases possibly linked to this outbreak following these alerts.

Discussion

Routine surveillance of paediatric HUS identified this outbreak of SF EHEC O157:[H7] in northern France due to foodborne transmission from contaminated ground beef preparations. While EHEC O157 is the serogroup most frequently isolated from French paediatric HUS cases [7], SF O157:[H7] had never been isolated from such patients in France prior to this outbreak (P. Mariani-Kurkdjian, personal communication).

Epidemiological, microbiological and trace-back investigations strongly suggest that products of brand 'A' produced by the producer 'E' and sold in supermarket chain 'X' were the outbreak source. Investigation results point to contamination of the raw beef from one of the three suppliers as the most likely source of this outbreak. The isolation of strains displaying nine distinct STEC pulsotypes from preparations produced on at least four different dates in May 2011 suggests widescale and probable heterogeneous contamination. This hypothesis is all the more likely as, although SF O157 is rarely isolated in food samples, two different pulsotypes (C and E) of SF O157 were found. Microbiology and trace-back investigation results suggest that the beef used for production on 11 May was particularly contaminated. The presence of multiple STEC pulsotypes in the beef would suggest a significant breach in hygiene procedures during its preparation and the presence of either a multiply contaminated cattle herd or multiple herds contaminated with different STEC serotypes.

Despite a wide-scale distribution of diverse ground beef preparations produced from the implicated batches of raw beef, identified cases were principally located in two administrative districts in northern France. Investigation results suggest that this geographical clustering could probably be explained by the distribution of 83% of the beef burgers produced on 11 May, the date on which the beef used for production was particularly contaminated, to stores of supermarket chain 'X' in the two districts where 77% of cases resided.

Interestingly, all strains of pulsotype 'E' found in beef and one found in case no.12 did not possess any *stx* genes whereas other strains of pulsotype 'E' isolated from patients were *stx*-positive. In EHEC strains, *stx* genes are typically harboured by transmissible lambdoid bacteriophages [20]. The fact that all these strains share an identical panel of EHEC auxiliary virulence factors and genetic background and are associated with clinical syndromes, supports the hypothesis that *stx*-negative strains are EHEC that lost *stx* genes. Indeed, the loss of

stx genes by *stx*-positive strains has been described *in vitro*, when strains are exposed to particular conditions, and *in vivo*, including during the course of infection [20,21]. We can infer that during the manufacturing process or the storage life of minced meat, this bacterial population undergoes stress [22] that might induce the lytic phase of the phages and excision of *stx*-converting bacteriophages from the EHEC genome. Another hypothesis would be that the loss of the *stx* genes arises during the sample processing or culture in the laboratory, as has been observed elsewhere [23].

An interesting feature of this outbreak was the absence of identified cases of bloody diarrhoea not complicated by HUS. Among the families of the 18 HUS cases identified, a single case of non-bloody diarrhea was reported. This observation is coherent with previously published SF O157:[H7] outbreak investigation results in Germany where active case-finding efforts and on one occasion an extensive search for diarrhoeal illness, including wide-scale stool screening in potentially exposed groups, identified very few cases of STEC gastroenteritis without HUS [3,24]. The authors of these investigations conclude that this feature could support the view of an increased virulence associated with SF O157:[H7].

The epidemiology of SF EHEC O157:[H7] infections is poorly understood and transmission routes and vehicles remain largely non-elucidated [1–3]. Suspected transmissions from food, person to person spread, the environment and contact with ruminants have previously been documented [1,24–26]. This outbreak confirms that ground beef can be a vehicle for foodborne transmission of SF EHEC O157:[H7]. Various food products, including locally produced apple-cider, quark cheese, sliced sausage ('mortadella' and 'teewurst') and raw cow's milk, have previously been implicated in SF O157:[H7] outbreaks but without microbiological confirmation [3,4,25].

While cattle are universally accepted as the major animal reservoir of classic EHEC O157:H7, the reservoir of SF O157:[H7] strains remains unclear [1,2]. The possibility of a human reservoir has also been proposed [1]. Evidence of an animal reservoir is rare, although some evidence of a cattle reservoir has been documented [1,27,28]. Despite causing *c.* 20% of sporadic HUS cases in Germany and multiple HUS outbreaks since 1988, SF O157:[H7] has rarely been identified from animals in Germany [1,29]. While we cannot exclude the possibility that the implicated raw beef in this outbreak was contaminated by another transmission route before transformation into ground beef products, our investigation could provide further evidence of an animal reservoir in cattle.

This outbreak was detected by routine surveillance of paediatric HUS. While it may seem less than optimal to use surveillance of a post-infectious syndrome for outbreak detection due to the associated time delay in diagnosis, this is another example of the outbreak detection capacity of HUS surveillance when STEC surveillance is not exhaustive [6,30,31].

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Authorship/Contribution

Lisa A King prepared the manuscript and all authors contributed to the writing of the sections that concern their area of expertise and critically read the manuscript. Lisa A King, Pascal Chaud, Henriette de Valk, Sylvie Haeghebaert and Véronique Vaillant conducted the outbreak investigation. Nathalie Pihier and Hélène Callon conducted the veterinary and trace-back investigation presented. Robert Novo provided the clinical information on the patients presented. Patricia Mariani-Kurkdjian, François-Xavier Weill, Edouard Bingen, Malika Gouali,

Charlotte Baliere and Olivier Gaillot conducted the human microbiological investigation presented. Estelle Loukiadis, Sarah Ganet and Delphine Thevenot-Sergentet conducted the food microbiological investigation presented and prepared human and food analysis results for presentation in Fig. 3.

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Transparency Declaration

The authors declare no conflicts of interest.

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