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Epidemiological analysis of a large enterohaemorrhagic *Escherichia coli* O111 outbreak in Japan associated with haemolytic uraemic syndrome and acute encephalopathy

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SUMMARY

A large outbreak of enterohaemorrhagic *Escherichia coli* (EHEC) O111 and O157 occurred in Japan in April 2011. We conducted an unmatched case-control study and trace-back investigation to determine the source of EHEC O111 infection and risk factors for severe complications. Pulsed-field gel electrophoresis was performed to help define cases. A total of 86 individuals met the case definition. Of these, 40% experienced haemolytic uraemic syndrome (HUS), 24% acute encephalopathy, and 6% died. Illness was significantly associated with eating the raw beef dish *yukhoe* (odds ratio 19·64, 95% confidence interval 7·03–54·83), the likely food vehicle. EHEC O111 and its closely related *stx*-negative variants were found in the beef. HUS occurred most frequently in individuals aged 5–9 years, and this age group was significantly associated with acute encephalopathy. The prevalence of HUS and acute encephalopathy was higher than in previous non-O157-related outbreaks, indicating a high risk of severe complications.

Key words: *Escherichia coli* infection, foodborne diseases, infectious disease outbreaks.

INTRODUCTION

Since the first reported case of human infection with Shiga toxin-producing enterohaemorrhagic *Escherichia coli* (EHEC) O157, there have been numerous reports of O157 and non-O157 EHEC outbreaks in a variety of settings (e.g. foodborne, human to human, waterborne, animal contact), with characteristic complications such as haemolytic uraemic syndrome (HUS) and acute encephalopathy (AE) [1–14]. In May 2012, the United States Department of Agriculture added six non-O157 serotypes (O26, O45, O103, O111, O121, O145) to the list of pathogens considered to be of public health importance [15]. In Japan, around 3000–4000 EHEC cases are reported annually and almost all are sporadic. EHEC O111 is the aetiological agent of about 4% of EHEC cases in Japan [16], and is responsible for 1–9 cases of HUS annually [National Epidemiological Surveillance of Infectious Diseases (NESID), unpublished data].

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HUS develops in around 3·2–4·0% (NESID, unpublished data) of all symptomatic EHEC cases in Japan, and about 90 HUS cases reported annually. Of the variants, EHEC O157 is the most prevalent, and EHEC O111 infection causes 1–9 cases of HUS annually in Japan (NESID, unpublished data). From 2006 to 2010, there were 83 EHEC outbreaks in Japan in which $\geqslant$10 EHEC-positive cases were reported. Of these, six outbreaks were due to EHEC O111 [17–21]: three due to EHEC O111 *stx1* and the other three due to EHEC O111 *stx1stx2*. Outbreaks due to EHEC O111 *stx2* are very rare in Japan.

Detection of the outbreak

On 26 April 2011, a general hospital in Toyama Prefecture, Japan informed a local public health centre (LPHC) of a suspected case of diarrhoeagenic *E. coli* infection. Several cases of *E. coli* O111 with *fliC*$_{H8}$ (*eae* positive and *aggR* negative with or without *stx2*) were reported on 27 April 2011 in Toyama, Fukui, and Kanagawa prefectures on 27 April 2011, and some of these cases showed severe complications such as HUS and AE for at least 1 day after onset [22, 23]. Two fatalities were reported in hospitals in Fukui and Toyama, on 28 and 29 April. Before symptom onset, all patients reported eating at one of 20 branches of barbecue restaurant chain A, although ultimately only six of the branches were involved in the outbreak. The restaurants serve mainly grilled meat (beef, pork, chicken) and raw beef that customers grill themselves. The chain also serves *yukhoe*, a seasoned raw beef rib dish similar to steak tartare, which was consumed by around 40% of customers (sales figure from the restaurant). On receipt of the information, Toyama prefectural government performed a risk assessment, which recognized that there may be additional cases outside the prefecture linked to the restaurant and HUS cases. On 4 May, Toyama Prefecture requested that the National Institute of Infectious Diseases, Japan, conduct an epidemiological investigation. The 20 branches of the chain are located across four prefectures (Toyama, Fukui, Ishikawa, Kanagawa).

According to preliminary information on the outbreak, EHEC O111 and/or *stx*-negative *E. coli* O111 were isolated from 68 cases. In addition to EHEC O111 and/or *stx*-negative *E. coli* O111, EHEC O157 was isolated from 18 patients. Although the impact of EHEC O157 in this outbreak was unclear, the findings of a serological study suggested that EHEC O111 might have played a primary role [24]. Thus, for our epidemiological investigation, we set a case definition for infection with EHEC O111 or its *stx*-negative variant. This report details the findings of the investigation into the source of infection and the risk factors for clinical complications, such as HUS and AE.

METHODS

Case and control definition and case-finding

EHEC is a notifiable disease in Japan. After being notified of a case by a physician, LPHCs conducted active case-finding in the contacts of patients and visitors to restaurant chain A. LPHC staff interviewed visitors and relatives of patients about their health status and collected faecal specimens from all contacted persons. Thereafter, LPHC staff continued to monitor each contact. A suspected case was initially defined as a gastrointestinal illness in a person who ate at the restaurant chain between 10 and 29 April 2011. LPHC staff also enrolled asymptomatic individuals who visited the restaurant chain together with a suspected case. Stool samples were collected from suspected case patients and their family and friends. A confirmed case was defined as at least one acute gastrointestinal illness, such as diarrhoea, bloody stool, abdominal pain, or vomiting, after visiting at least one branch of the restaurant chain during the observation period (until 10 May), with laboratory confirmation through bacterial isolation of *E. coli* O111 (*stx* or *eae* gene-positive) or a positive result for the anti-*E. coli* O111 antibody by microagglutination assay at the Toyama Institute of Health (THI) and/or the National Institute of Infectious Diseases (NIID). The anti-*E. coli* O111 antibody assay was conducted on stored serum from both HUS and non-HUS hospitalized cases. A secondary infection was defined as that arising after contact with a case at home, school, or work and with no history of visiting the restaurant chain. We excluded secondary cases from the analysis. Subjects with insufficient clinical, demographic, or laboratory data were also excluded. HUS was defined by the presence of at least two of the following: (*a*) haemolytic anaemia, (*b*) thrombocytopenia (platelet count $\leqslant$150 000/mm^3), and (*c*) acute renal dysfunction [defined by at least one of the following: reduced renal function (e.g. increased serum creatinine), oliguria (reduced urinary excretion, <500 ml/24 h), renal failure (e.g. anuria; urinary excretion <100 ml/24 h), proteinuria, and haematuria] [25]. AE was defined as follows: (*a*) *confirmed*, at

least one neurological symptom (e.g. speech or behavioural abnormalities, or seizure) persisting for at least 12–24 h and abnormal brain imaging findings on magnetic resonance imaging (MRI) or computed tomography; or (*b*) *suspected*, at least one neurological symptom. A control was defined as any individual who visited a branch of the restaurant chain between 10 and 29 April and had no gastrointestinal symptoms or evidence of infection with *E. coli* O111.

Study design

The outbreak investigation included an unmatched case-control study and trace-back investigation. The case-control study was conducted to identify the sources of infection associated with illness and the risk factors associated with severe complications. The trace-back investigation was conducted by the LPHCs and included laboratory examinations of common menu items available at all branches of the restaurant chain.

Logistic regression analysis was used to identify sources of infection and risk factors for HUS and AE. Adjusted odds ratios obtained from multivariate logistic regression were adjusted for *yukhoe* consumption. Statistical analyses were conducted with SAS v. 9.2 (SAS Institute Inc., USA).

Epidemiological information, including demographic information and information on risk factors, such as the consumption of specific menu items, was gathered by 27 LPHCs. The medical records of all cases from 26 hospitals were reviewed and clinical and laboratory data collected.

Laboratory tests

Stool samples and sera were collected from patients, and bacterial culturing of stool samples was performed by the LPHCs and TIH. Determination of the flagellar antigen type of the EHEC O111 outbreak strain was conducted by *fli*C typing by polymerase chain reaction/restriction fragment length polymorphism [22, 26]. Serological testing for anti-*E. coli* O111 antibodies was conducted mainly in HUS cases by the TIH and/or NIID. Isobe *et al.* [24] reported that the antibody to *E. coli* O111 is considered negative in control sera. Antibody testing was performed in HUS cases to diagnose O111 infection because antibodies to EHEC O111 are generally absent in the general Japanese population. Therefore, we suspect that the Japanese population might not have pre-existing immunity for *E. coli* O111. Pulsed-field gel electrophoresis (PFGE) analysis was performed as described previously [27]. The resulting PFGE patterns were analysed using BioNumerics v. 6.6 software (Applied Maths, Belgium).

Trace-back and trace-forward investigations

The public health authority of Toyama Prefecture requested that the LPHCs conduct a trace-back investigation of all food suppliers and processing companies of restaurant chain A. The public health authority conducted a trace-forward to ensure that all suspected contaminated food materials were removed from the market, according to the preliminary epidemiological investigation.

Ethical statement

This outbreak investigation, case-control study, and trace-back investigation was conducted in accordance with the Act on Prevention of Infectious Diseases and Medical Care for Patients Suffering Infectious Diseases and the Food Sanitation Act.

The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008.

RESULTS

Case findings

The LPHCs collected the data of 941 individuals who reportedly visited the restaurant chain between 10 and 29 April 2011. Of these, 326 (35%) developed at least one gastrointestinal symptom and were classified as suspected cases (Fig. 1), while 86 met the case definition for *E. coli* O111 infection and were classified as confirmed cases. Of these 86 cases, *E. coli* O111 was isolated in 69 (80%) and the remaining 17 (20%) cases were anti-*E. coli* O111 antibody-positive. Of the 326 symptomatic cases, 210 were negative for *E. coli* O111 and 17 did not seek medical care. Two patients were household contacts of case-patients and were excluded as secondary infections. Of the 615 individuals who showed no gastrointestinal symptoms, 325 met the definition for a control. Of the 290 individuals excluded as controls, 284 could not be interviewed in detail, and EHEC O157 was isolated in six (asymptomatic cases).

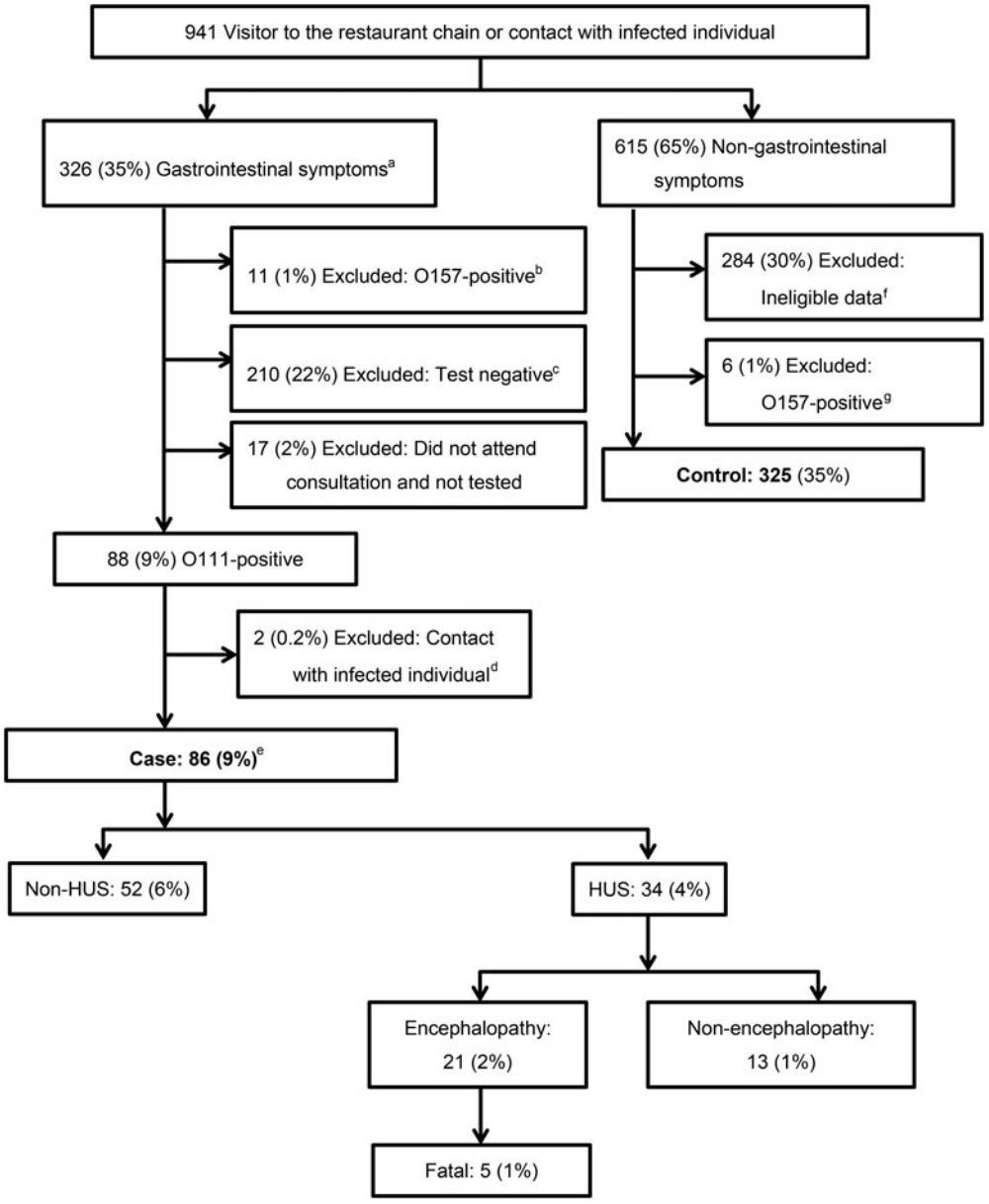

Fig. 1. Flowchart of cases and controls in the outbreak investigation. [a] Suspected cases; [b] EHEC O157 isolated only; [c] tested negative for EHEC O111, EHEC O157, and other agents; [d] secondary infection cases; [e] of 86 cases, 18 were dual infection with EHEC O111 and O157; [f] could not be interviewed in detail; [g] asymptomatic cases.

Illness onset occurred between 19 April and 4 May 2011 (Fig. 2*a*) in all 86 case-patients, with peaks on 25 and 26 April. No additional cases were reported after 4 May. The median incubation period was 3 days (range 1–11 days) from the time of exposure in one of the restaurants to gastrointestinal symptom onset. The restaurant exposure dates were 17–25 April, with a peak on 23 April. The number of patients visiting different branches was: branch A ($n = 60$), branch B ($n = 14$), branch C ($n = 3$), branch D ($n = 1$), branch E ($n = 7$), and branch F ($n = 1$) (Fig. 2*a*). Of the 86 cases, 51% were women and the median age was 20·5 years. The most affected age group was 15–19 years (26%), followed by 20–24 years (14%) (Table 1). Most of the 86 patients developed diarrhoea (95%) or bloody stool (55%). The rate of isolation of *E. coli* O111 was 80%, and the detection rate of *E. coli* O111 antibody alone was 20%. Of the 86 patients, 46 (53%) were hospitalized, 34 (40%) were diagnosed with HUS, 21 (24%) were diagnosed with AE, and the case-fatality rate was 6% (5/86). The median period from exposure to symptom onset for HUS

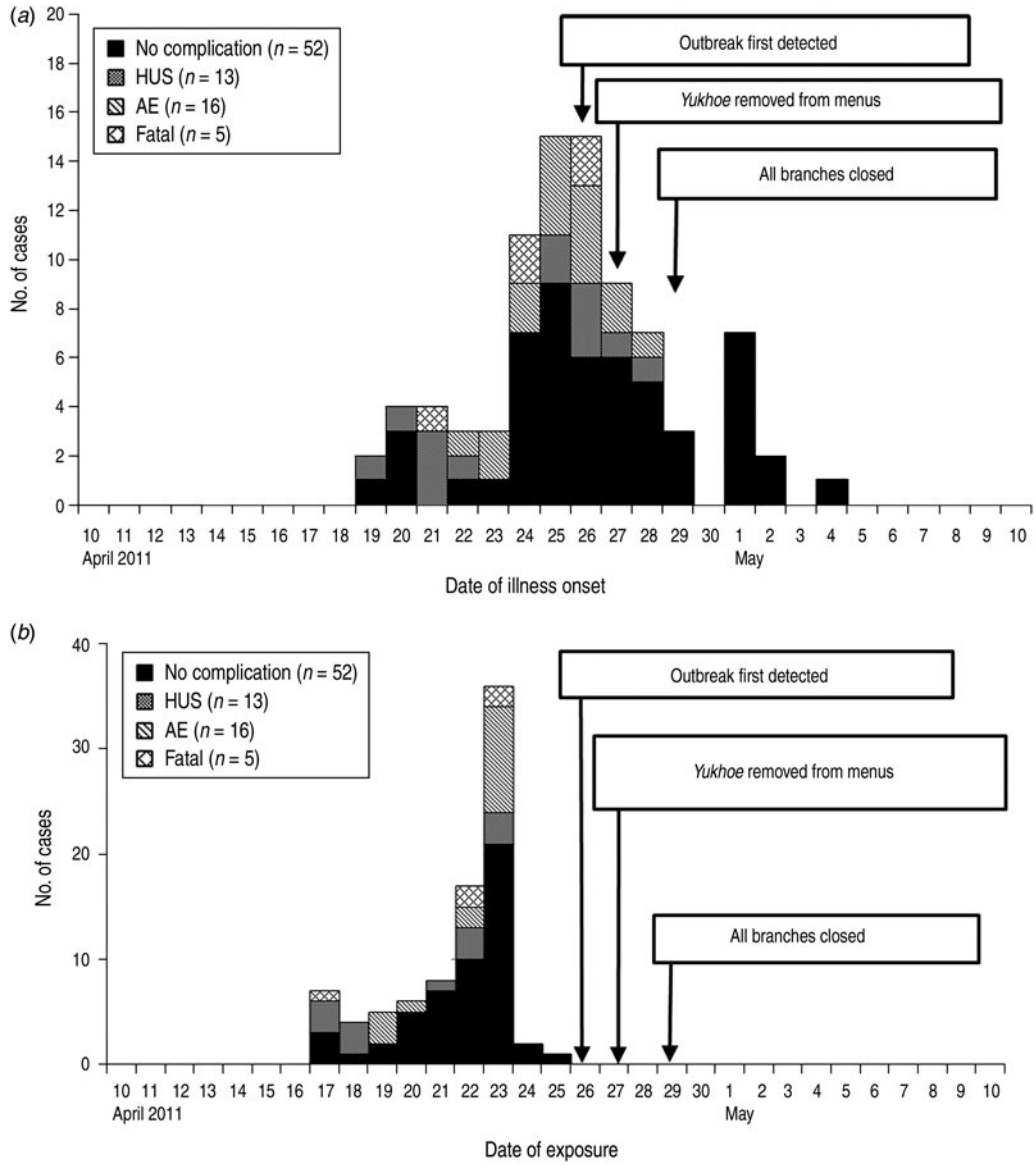

Fig. 2. Number of cases by (*a*) date of illness onset with outcome and (*b*) date of exposure in Toyama, Fukui, Ishikawa, and Kanagawa prefectures in 2011 ($n = 86$).

cases (3 days) was significantly shorter than that for non-HUS cases (4 days) ($P = 0{\cdot}011$). All AE cases were associated with HUS, and all fatal outcomes were associated with AE.

Analytical case-control study

There was no difference in sex distribution between case subjects and controls (Table 1). The median age was 20·5 years for cases and 27·5 years for controls ($P = 0{\cdot}177$). Ninety-five percent of the 86 case-patients reported consuming *yukhoe* compared to 51% of controls (Table 2). Beef rib and beef tongue were the second and third main food items, respectively, that were consumed by both the case and control groups. Two patients aged <4 years had not consumed *yukhoe* but their parents had. There was no difference in *yukhoe* consumption between men (97%) and women (93%) ($P = 0{\cdot}284$). Excluding those aged 0–4 years, 75–100% of case-patients and 36–67% of controls consumed *yukhoe* in each age group. For controls, *yukhoe* consumption was the most common item in individuals aged 10–14 years (76%), followed by those aged 15–19 years (67%). *Yukhoe* consumption was significantly higher in case patients than controls for all age groups except those aged 10–14 years, 20–24 years, and >50 years. By

Table 1. *Characteristics of subjects*

	Case patients ($N = 86$)		Controls ($N = 325$)	
	n	%	*n*	%
Sex*				
Male	42	49	149	48
Female	44	51	159	52
Total	86		308	
Age group (years)†				
0–4	2	2	26	11
5–9	9	10	22	10
10–14	8	9	21	9
15–19	22	26	18	8
20–24	12	14	16	7
25–29	11	13	21	9
30–39	11	13	48	21
40–49	7	8	33	14
⩾50	4	5	25	11
Total	86	100	230	100
Symptom				
Diarrhoea	82	95		
Bloody stool	47	55		
Abdominal pain	75	87		
Nausea	22	26		
Vomiting	26	30		
Fever	35	41		
Seizure	8	9		
Headache	8	9		
E. coli O111 isolation/ antibody testing				
Isolation only	43	50		
E. coli O111 isolation and anti-*E. coli* O111 antibody-positive	26	30		
Anti-*E. coli* O111 antibody-positive only	17	20		
Complication and fatality				
HUS	34	40		
Acute encephalopathy	21	24		
Fatal outcome	5	6		

HUS, Haemolytic uraemic syndrome.
* Sex of controls: data missing in 17 cases.
† Age group of controls: data missing in 95 cases.

univariate analysis (Table 2), illness was significantly associated with consumption of *yukhoe* [odds ratio (OR) 19·64, 95% confidence interval (CI) 7·03–54·83] and beef diaphragm muscle (OR 2·13, 95% CI 1·29–3·51). *Yukhoe* consumption adjusted by age was also significantly associated with illness (OR 21·95, 95% CI 7·71–62·45). After adjustment for *yukhoe* consumption, the remaining food items were not significantly associated with illness.

Risk factors for complications

Among the 86 confirmed cases, 34 developed HUS and 21 developed AE, including seven suspected cases of AE. The median period from symptom onset to development of HUS was 4 days (range 0–9 days). The time from HUS to AE was not significantly different between confirmed AE (median 1 day, range 0–4 days) and suspected AE (median 3 days, range 0–7 days) ($P = 0·176$). The time from the development of neurological symptoms to brain imaging in suspected cases (median 3 days, range 0–6 days) was significantly longer than that in confirmed AE cases (median 0 days, range 0–9 days) ($P = 0·015$). Clinical and radiographic descriptions of the AE cases have been reported in detail by Takanashi *et al.* [23].

A significantly higher proportion of female patients developed HUS and AE than male patients (Table 3). Of individuals aged <40 years, those aged 5–9 years showed the highest rate of HUS (67%), followed by the 15–19 years age group (59%). The highest rates of AE occurred in the 5–9 years (56%) and 0–4 years (50%) age groups. By univariate analysis, female sex was significantly associated with the development of HUS (OR 2·50, 95% CI 1·02-6·10) and AE (OR 3·10, 95% CI 1·07-9·01). The reference age group with the lowest rate of HUS was 30–39 years. The development of HUS was significantly associated with the 5–9 years (OR 16·48, 95% CI 1·29-1008·47) and 15-19 years (OR 13·35, 95% CI 1·45-673·80) age groups, while the development of AE was significantly associated with those aged 5–9 years (OR 13·89, 95% CI 1·56-∞).

Microbiological investigation

The *stx*-negative strains isolated from patients showed similar PFGE patterns, with two bands differentiating them from EHEC O111 with $fliC_{H8}$ isolates (Fig. 3). This indicated that these *E. coli* O111 strains were closely related. Furthermore, EHEC O111 and its *stx*-negative variants were isolated from a stored beef rib meat block (the raw material of *yukhoe*) in branch F. Based on the size of each unique band of *stx2*-producing and *stx*-negative strains, differences in the PFGE patterns were consistent with the presence or absence of *stx2* phage. Two distinct strains, *stx2*-positive and -negative strains, were isolated from the suspected beef rib meat block. These *stx2*-positive and -negative strains were indistinguishable from the strains isolated from the cases. The

Table 2. *Association between food intake and onset of illness**

	Cases ($N = 86$)		Controls ($N = 325$)			
	n	%	*n*	%	Crude OR (95% CI)	Adjusted† OR (95% CI)
Raw or grilled meat						
Yukhoe (raw beef)	82	95	166	51	19·64 (7·03–54·83)	–
Beef rib	61	71	86	26	0·89 (0·53–1·51)	0·41 (0·22–0·78)
Beef tongue	41	48	134	41	1·30 (0·81–2·09)	0·88 (0·53–1·47)
Beef small intestine	13	15	63	19	0·74 (0·39–1·42)	0·52 (0·26–1·03)
Beef diaphragm muscle	35	41	79	24	2·13 (1·29–3·51)	1·62 (0·95–2·77)
Chicken coccyx meat	25	29	54	17	2·06 (1·19–3·56)	1·59 (0·88–2·86)
Vegetables						
Bean sprouts	6	7	16	5	1·45 (0·55–3·82)	1·17 (0·42–3·29)
Korean pickles	15	17	43	13	1·39 (0·73–2·64)	1·14 (0·57–2·25)
Lettuce	13	15	58	18	0·82 (0·43–1·58)	0·77 (0·38–1·53)
Salad	6	7	18	6	1·28 (0·49–3·33)	1·16 (0·41–3·22)
Organic lettuce	1	1	10	3	0·37 (0·05–2·94)	0·19 (0·02–1·53)
Caesar salad	16	19	60	18	1·01 (0·55–1·86)	0·97 (0·51–1·87)
Sliced tomato‡	2	2	11	3	0·68 (0·15–3·13)	0·48 (0·10–2·31)
Other						
Cold noodles	24	28	60	18	1·71 (0·99–2·96)	1·07 (0·60–1·92)

OR, Odds ratio; CI, confidence interval.
* Of the 47 items, 33 were not significantly associated with EHEC O111 infection (data not shown).
† Adjusted by *yukhoe* consumption.
‡ Missing data: one control.

details of the microbiological characterization of EHEC O111 and its *stx2*-negative variant have been reported previously [22].

Trace-back and trace-forward investigation

The LPHCs performed trace-back investigations of the suppliers of all food items served with the meat at the restaurant; the suppliers of each branch varied, but nine food companies in total covered all branches. From 1 to 29 April 2011, the chain served 47 food items; *yukhoe* and seven other items were common to all branches. In each branch, *yukhoe* was made from several blocks of chopped beef rib meat, which was cut several centimetres thick, seasoned, served in pâté form, and eaten without cooking. All beef rib was from a domestic farm and was processed at a single plant. We could not determine the source of cattle by identification numbers of the beef rib: neither the meat processing company nor the restaurant chain recorded these details. Common grilled meat items included beef flank, beef tongue, and beef small intestine served with Korean lettuce, Korean pickles, and seasoning sauce. *Yukhoe* was processed in compliance with the operation manual, from cutting the meat to serving the dish. The meat processing company did not distribute the same beef product to other companies.

Public health control measures

On 27 April 2011, Toyama prefectural government and an LPHC recommended that the restaurant chain stop serving *yukhoe*. On 29 April, after a fatal case was reported, all branches were temporarily closed.

On 5 May 2011, the Ministry of Health, Labour, and Welfare (MHLW), Japan took emergency measures to stop the serving of raw beef in all restaurants. Furthermore, on 1 October 2011, the MHLW implemented a standardized process for serving *yukhoe* under the Food Sanitation Act; on 7 July 2012, this law also restricted the serving of raw beef liver. The revised standardized process requires meat to be heated to at least 60 °C for 2 min to kill microorganisms. After this process, each side trimmed from the meat may be used to make *yukhoe*.

EHEC O157 cluster

Of the 86 cases, EHEC O157 was isolated from 18 patrons of the restaurant chain. Of these 18 cases,

Table 3. *Relationship between sex, age, symptoms, and serious complications (HUS or AE)*

	Cases	HUS			AE			Fatal	
	N	*n*	%	HUS *vs.* non-HUS OR (95% CI)	*n*	%	AE *vs.* non-AE OR (95% CI)	*n*	%
Sex									
Male	42	12	29	1·00 (reference)	6	14	1·00 (reference)	3	14
Female	44	22	50	2·50 (1·02–6·10)	15	34	3·10 (1·07–9·01)	2	34
Total	86	34	40		21	24		5	24
Age group (years)									
0–4	2	1	50	7·42 (0·06–860·49)	1	50	5·50 (0·14–∞)		
5–9	9	6	67	16·48 (1·29–1008·47)	5	56	13·89 (1·56–∞)	2	22
10–14	8	3	38	5·43 (0·34–345·69)	3	38	6·73 (0·64–∞)	1	13
15–19	22	13	59	13·35 (1·45–673·80)	6	27	5·08 (0·64–∞)		
20–24	12	5	42	6·57 (0·56–370·82)	1	8	0·92 (0·02–∞)		
25–29	11	2	18	2·15 (0·10–143·71)	2	18	2·59 (0·19–∞)		
30–39	11	1	9	1·00 (reference)	0	0	1·00 (reference)		
40–49	7	1	14	1·62 (0·02–143·71)	1	14	1·57 (0·04–∞)	1	14
⩾50	4	2	50	8·08 (0·30–646·82)	2	50	8·42 (0·59–∞)	1	25
Total	86	34	40		21	24		5	29
Symptom									
Diarrhoea	82	34	43	3·63 (0·44–∞)	21	26	1·76 (0·21–∞)	5	100
Bloody stool	47	32	68	37·49 (7·97–362·10)	20	43	27·27 (3·90–1196·51)	5	100
Abdominal pain	75	34	45	12·25 (1·89–∞)	21	28	5·76 (0·87–∞)	5	100
Nausea	22	18	82	13·01 (3·60–60·82)	11	50	5·27 (1·61–18·03)	2	40
Vomiting	26	22	85	20·94 (5·73–99·70)	15	58	11·79 (3·44–46·25)	4	80
Fever	35	22	63	7·50 (2·57–23·76)	19	54	11·24 (3·24–46·25)	5	100
Seizure	8	8	100	20·95 (3·07–∞)	8	100	50·12 (7·16–∞)	4	80
Headache	8	5	63	2·78 (0·50–19·23)	1	13	0·42 (0·01–3·59)		

HUS, Haemolytic uraemic syndrome; AE, acute encephalopathy; OR, odds ratio; CI, confidence interval.

nine developed HUS. After excluding 18 cases of dual infection, a repeat of the analysis did not change the results substantially. An additional 11 cases who were not included in the 86 cases were EHEC O157 culture-positive (not EHEC O111 or its *stx2*-negative variant), but these 11 cases did not progress to HUS. The source of EHEC O157 was not confirmed and EHEC O157 was not isolated from the beef rib meat.

DISCUSSION

This outbreak investigation examined epidemiological, microbiological, and food trace-back and trace-forward data and it implicated *yukhoe* beef contaminated with EHEC O111 and its *stx*-negative variant as the source of the outbreak. The case-control study found a strong association between *yukhoe* consumption and illness, and no association with other foods. Moreover, EHEC O111 and its *stx*-negative variant were isolated from leftover beef. According to the trace-back investigation, the raw meat material of *yukhoe* was distributed by a single meat processing company to all branches of the restaurant chain. *Yukhoe* was removed from the menu the day after the outbreak was officially recognized and the restaurants were closed after 3 days. These measures probably prevented additional cases because the contaminated meat was, until that point, still being served, at least at branch F.

In this outbreak, *yukhoe* consumption is the likely source of the EHEC O111 infections. However, 51% (166/325) of the control group also consumed *yukhoe*. It is possible that some EHEC O111 isolates in this outbreak easily lost the *stx2* phage and converted to the *stx2*-negative variant [22]. This could suggest that the dose of EHEC O111 involved in the contamination was relatively low, although we were unable to determine amounts.

In the USA, EHEC O111 is not often a cause of HUS [28]. However, an outbreak in Oklahoma caused by EHEC O111 included 11 HUS cases; the proportion of development was as high as 17% in confirmed or probable cases and 7% in cases of gastrointestinal illness without bacteriologically positive

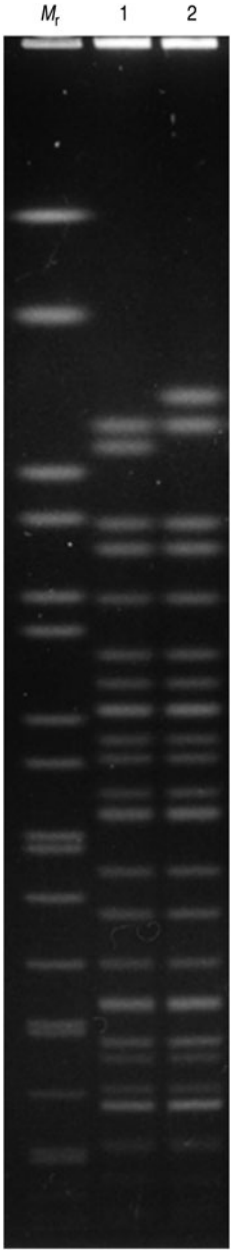

Fig. 3. Pulsed-field gel electrophoresis analysis results. M_r, DNA size marker, *Salmonella* Braenderup strain H9812. Lane 1, patient 1, O111:H$^-$, VT1–, VT2–. Lane 2, patient 2, O111:H$^-$, VT1–, VT2+.

results [13, 29]. EHEC O104 caused another outbreak with a high HUS rate of 22% in Germany [30]. This high rate of HUS development may have been due to an atypical strain of EHEC, which has now been characterized (*stx2* positive, *aggR* positive, *eae* negative). Although our outbreak strain was a typical EHEC (*stx2* positive, *eae* positive, *aggR* negative), the proportion of HUS was 40% in confirmed cases and 10% in suspected cases, which is higher than that of the Oklahoma EHEC O111 outbreak [23, 29]. Since the outbreak in this study was caused by typical EHEC O111, we sought reasons for the higher than usual proportion of HUS.

There are several possible explanations for this. Cases may have consumed a greater dose of *E. coli* O111 STEC than controls because they ate a greater quantity of *yukhoe* or because the distribution of *E. coli* O111 in the *yukhoe* was not uniform; however, we did not measure the amount eaten or quantify the level of food contamination. It is also possible that EHEC O111 in some parts of the *yukhoe* lost the *stx*2 phage, or that mild cases were undetected. However, including the suspected cases ($n = 326$), the proportion of HUS development was high. Alternatively, the strain of EHEC O111 might have been more virulent, such as a high producer of *stx2*, although there was no evidence of this.

One of the remaining unevaluated issues in this study is that this outbreak was caused by both EHEC O111 and EHEC O157. When we set the other definitions for EHEC O157 infection, we could not obtain a clear indication of the source (data not shown). Of the patients who were culture-positive for both EHEC O111 (or its *stx2*-negative variant) and EHEC O157, the rate of HUS development was higher than that of a previously reported group [22]. Our findings do not support the theory that EHEC O157 was the major contributor to the development of HUS because the rate of HUS development in the EHEC O157-only culture-positive cases was low (1/18 in Watahiki *et al*. [22]). At this point, it is not clear why a doubly-positive patient developed severe complications.

Our data showed that the risk factors for complications were female sex and age groups 5–9 and 15–19 years. Another investigation also indicated that female sex was associated with the development of severe complications [31]. The implications of this association may point to a sex difference in Gb3 (*stx* receptor) on human cells. We attempted to analyse HUS stratified by sex and age group but were unsuccessful due to the small numbers in each group.

The factors significantly associated with HUS and AE include sex, bloody stool, nausea, and vomiting (Table 3). Moreover, in this outbreak investigation symptoms such as fever, seizure, and headache were also significantly associated with diagnoses of HUS and AE. In previous *E. coli* outbreaks, sex and bloody stool were significantly associated with HUS [13, 31–34]. One of the strengths of this outbreak

investigation was that we obtained detailed information about symptoms. Consequently, the clinical outline of severe complications could be obtained via pathological imaging findings (MRI or computed tomography). In this study, we were able to collect detailed information and laboratory results for all 86 confirmed cases, enabling us to clarify the risk factors for the source of infection and severe complications of EHEC O111 and/or its *stx*-negative variants infection.

This study used case-control analysis and as such there is some susceptibility to recall bias. In addition, although elaborate bacteriological testing was conducted in laboratories and by the TIH, some cases may have been unconfirmed due to a lack of laboratory testing in the isolation of *E. coli* O111, because it is currently difficult to isolate EHEC in routine laboratory settings, especially in the case of *stx*-negative variants. Thus, there may have been some misclassification of cases and controls.

In conclusion, our investigation linked an outbreak of 86 cases that met the case definition to the consumption of raw beef contaminated with EHEC O111. Severe complications such as HUS and AE occurred not only in young children (aged <5 years), but also in other age groups. Severe complications such as HUS and AE and the case-fatality rate were much higher in this outbreak. Moreover, the majority of HUS and AE cases occurred in patients aged 5–24 years and female patients. This outbreak exhibited not only gastrointestinal illness, but also high rates of HUS and AE. The MHLW implemented a revised standardized process for serving *yukhoe* and a restriction on the serving of raw beef liver by law. To prevent illness, citizens need to be advised about the contagious agents that can contaminate raw beef, and its consumption should be discouraged.

APPENDIX

Additional members of the *E. coli* O111 Outbreak Investigation Team who contributed data: The physicians, laboratory personnel, and medical processor of Tonami General Hospital, Toyama University Hospital, Toyama City Hospital, Toyama Prefectural Central Hospital, Toyama Red Cross Hospital, Shinseikai Toyama Hospital, Takaoka City Hospital, Saiseikai Takaoka Hospital, Shakaihoken Takaoka Hospital, Kouseiren Takaoka Hospital, Nanto Municipal Hospital, Hokuriku Central Hospital, Kamiichi General Hospital, Imizu Municipal Hospital, Kanazawa University Hospital, Kanazawa Medical University Hospital, Himi Municipal Hospital, University of Fukui Hospital, Fukui Red Cross Hospital, Nittazuka Medical Welfare Center, Fukui Saiseikai Hospital, Imadate Central Hospital, Sendai City Hospital, Seirei Yokohama Hospital, Yodogawa Christian Hospital, and Tsuda Ladies & Maternity Clinic; Dr Hidemasa Izumiya and Dr Tomoko Morita-Ishihara of the Department of Bacteriology I, National Institute of Infectious Diseases.

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DECLARATION OF INTEREST

None.

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