

Epidemiological and microbiological investigation of a large outbreak of monophasic *Salmonella* Typhimurium 4,5,12:i:- in schools associated with imported beef in Poitiers, France, October 2010

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An outbreak due to the emerging monophasic *Salmonella* Typhimurium 4,5,12:i:- occurred in four schools in Poitiers in October 2010. Food trace-back investigation led to the identification of beef burgers as the cause of the outbreak and their subsequent withdrawal. The Institute for Public Health Surveillance conducted a retrospective epidemiological investigation to assess the extent of the outbreak and describe cases. Self-administered questionnaires were completed by students and personnel attending each of the four schools affected. Clinical cases were defined as anyone having eaten at the school when the beef burgers were served and reporting diarrhoea or fever with at least one digestive symptom (nausea, vomiting or abdominal pain), within five days after the incriminated school meal or with unknown date of onset within a 15-day period after the incriminated school meal. Of 1,559 persons exposed, 554 clinical cases were identified corresponding to an overall attack rate of 35.5%. Of 554 clinical cases, a total of 286 (53%) sought medical care and 31 (6%) were hospitalised for more than 24 hours. This multi-school outbreak is one of the biggest food-borne outbreaks of monophasic *Salmonella* Typhimurium 4,5,12:i:- described in France. Prompt notification of cases and rapid identification and withdrawal of the incriminated batch of beef burgers was crucial to limit the extension of this outbreak.

Introduction

In France, during the period 2006–2008, *Salmonella* was the cause of 23% of dispersed food-borne outbreaks [1]. Monophasic *Salmonella* Typhimurium 4,5,12:i:-, an emerging variant of serotype Typhimurium, is responsible for an increasing number of food-borne outbreaks in Europe [2-4]. The prevalence of this serotype among human salmonellosis cases has increased considerably since the mid-1990s. In the first decade of

the 2000s, this serotype represented one of the most common serotypes among human cases in many countries around the world [5-8]. In France, the incidence of serotype 4,5,12:i:- increased in the past decade and has become the third most common serotype identified in humans since 2008 [9]. In 2010 and 2011, the subspecies *enterica* serotype 4,5,12:i:- was identified in two nationwide outbreaks in France linked to dried pork sausage [2,10]. Data on the severity of clinical illness for those infected with serotype 4,5,12:i:- are still limited and reported hospitalisation rates vary among studies [4,11].

In October 2010, the regional health agency, the local Food Control Unit and the regional office of the Institute for Public Health Surveillance investigated a food-borne outbreak involving about 50 known cases attending four schools of Poitiers. The first eight cases were notified during school holidays. Monophasic *Salmonella* Typhimurium 4,5,12:i:- was isolated from stool samples of these first cases. Investigations by the Food Control Unit quickly identified frozen beef burgers produced in another European Union (EU) Member State as the cause of the outbreak. The meat originated from a single batch that was served for lunch in the first two schools reporting cases. Two days after notification of the first cases and without awaiting microbiological results on the products, the national food safety authorities (DGAL) asked the distributor to stop all deliveries to its clients (schools and restaurants). Four days later, DGAL initiated the withdrawal of all frozen beef burgers batches (22 batches in total) with the same production date as the incriminated batch.

As the *Salmonella* serotype identified is an emerging one in France and in Europe, the regional health

TABLE 1Study participation by school, *Salmonella* outbreak, Poitiers, France, October 2010

School	Persons in the school (including personnel)	Questionnaires received and participation rate in schools	Participants exposed to meal ^a	Meals distributed	Participation rate of persons exposed to meal
	n	n (%)	n	n	%
A	560	515 (92)	268	250	>100 ^b
B	922	838 (91)	712	752	95
C	687	524 (76)	449	578	78
D	554	254 (46)	130	226	58
Total	2,723	2,131 (78)	1,559	1,806	86

^a Persons who ate lunch on 22 October at Schools B, C, D and on 19 October at School A.

^b More participants than meals served.

authorities and the Institute for Public Health Surveillance decided to conduct an in-depth analysis of the outbreak and to measure its extent. This article describes the epidemiological and microbiological investigations undertaken to estimate the total number of cases involved in the outbreak of monophasic *Salmonella* Typhimurium 4,5,12:i:- in the schools of Poitiers and to describe their characteristics.

Methods

Epidemiological investigation

Twelve days after the initial alert, the regional office of the Institute for Public Health Surveillance conducted a retrospective epidemiological investigation in the four schools of Poitiers where the incriminated batch of frozen beef burgers had been served and where at least one case of gastrointestinal disease was reported.

We obtained the weekly menus of food served at the schools directly from the kitchen supervisors in order to know at which day(s) the incriminated beef was served and to have confirmation that there was only one single type of meal served per day.

A self-administered questionnaire was distributed to all students and personnel of the four schools. The questionnaire focused on consumption of a school meal on the day(s) the incriminated beef was served, age and sex, timing of illness, clinical symptoms and treatment. As the questionnaire was sent nearly two weeks after the date the incriminated beef was served, we asked persons interviewed about the date they ate lunch at school instead of asking them about specific food items.

A clinical case was defined as a person having eaten the school meal on the day the incriminated beef was served and reporting either: (i) diarrhoea within five days after school meal, or (ii) fever with at least one digestive symptom (nausea, vomiting or abdominal

pain) within five days after school meal, or (iii) diarrhoea of unknown date of onset but within 15 days after the incriminated school meal, or (iv) fever with at least one digestive symptom and with unknown date of symptoms within 15 days after the school meal. Confirmed cases were clinical cases with a positive stool culture for monophasic *Salmonella* Typhimurium 4,5,12:i:- as determined by the French National Reference Centre for *Salmonella* (NRC).

In order to calculate the participation rate, the number of meals delivered on the day(s) the contaminated beef was served was used as a proxy value for the number of individuals exposed to the incriminated meal. Only individuals who reported having eaten the school lunch on the day the incriminated beef was served were considered to be exposed. We assume that all those who ate at the school consumed the beef as only one type of meal is served for lunch on a given day.

Attack rates for disease and their 95% confidence intervals (CI) were calculated by age, sex, and school. Statistical analyses were conducted in STATA 10 (StataCorp, Tx).

This investigation was conducted with the authorisation of the French regulatory authority (Commission nationale de l'informatique et des libertés, request number 34.11.94 related to outbreak investigation).

Microbiological investigations

The Food Control Unit collected frozen beef burger from one school and cooked beef burger from a sample meal from a second school. Concentration of bacterial inoculum was measured by the Laboratory for Food Safety (ANSES).

Twenty-five human *Salmonella* isolates collected between 25 and 31 October 2010 by the laboratories of Poitiers and the two food isolates were sent to the NRC in Paris for serotyping, subtyping and

TABLE 2Characteristics of the exposed population, *Salmonella* outbreak, Poitiers, France, October 2010 (n=1,559)

School	A	B	C	D
Sex-ratio (male/female) ^a	1.0	1.0	0.9	2.3
Adults (≥20 years-old) ^b				
Number of exposed	11	33	7	14
Number of clinical cases	0	3	3	4
Adolescents (<20 years-old) ^b				
Number of exposed	254	671	433	116
Number of clinical cases	93	326	69	46
Median age in years (interquartile range) ^b				
Adults (≥20 years-old)	43 (38–53)	40 (34–51)	41 (28–45)	39.5 (29–51)
Adolescents (<20 years-old)	13 (11–13)	12 (11–13)	12 (11–13)	16 (15–17)

^a As 21 questionnaires missed information on sex, 1,538 questionnaires were used,

^b For cases whose age was known. As 20 questionnaires missed information on age, 1,539 questionnaires were used.

antimicrobiological susceptibility analysis. Subtyping of the isolates was carried out by standardised *Xba*I-pulsed-field gel electrophoresis (PFGE) and by multi-locus variable-number tandem repeat analysis (MLVA), as previously described [2,12]. The MLVA profiles were expressed according to the nomenclature published by Larsson et al [13]. The antimicrobial susceptibility testing to 32 antimicrobials was performed by disk diffusion on Mueller-Hinton agar according to the guidelines of the Antibiogram Committee of the French Society for Microbiology. Human isolates were compared with isolates from food using the same typing methods.

Results

Epidemiological results

From the 2,723 questionnaires distributed, 2,139 questionnaires were received (78.5%), from which eight were excluded from analysis because school information was missing.

A total of 1,559 participants declared having eaten at school on the days the incriminated beef burgers were served. Based on number of meals distributed on the day(s) the incriminated beef burgers were served (n=1,806), this represented a study participation rate of 86% across all schools for persons exposed (Table 1).

Three schools (Schools A, B and C) were Junior high schools (with 4 different levels) and one school (School D) was a Senior high school (3 higher levels). Median age of adolescents (<20 years-old) attending the school was higher in School D (median age of 16 years, range 14–19) than in the other schools (median age of 12–13 years, range 10–16).

All schools were mixed (Table 2). The sex-ratio of study participants was 1.0. School D had a majority of male students (sex-ratio: 2.3) (Table 2).

Exposed adults (≥20 years-old) represented 11 of 265 (4%) of all exposed in School A, 33 of 704 (5%) in School B, and seven of 440 (2%) in School C (Table 2), and were mainly personnel of the school. Adults in School D represented 14 of 130 exposed (11%) and included both personnel and students.

The epidemic curve of clinical cases per school is shown in the Figure. Only cases who reported the date of onset of their illness are included (n=493). The peak of the outbreak in School A occurred two days after the incriminated lunch was first served. At this school, the incriminated beef burgers were served on 19 October, and 25 additional beef burgers were also served on the following day. The peak of the outbreak in Schools B and D occurred one day after the incriminated lunch was served, and in School C, two days after. In the three later schools, the incriminated lunch was served on 22 October.

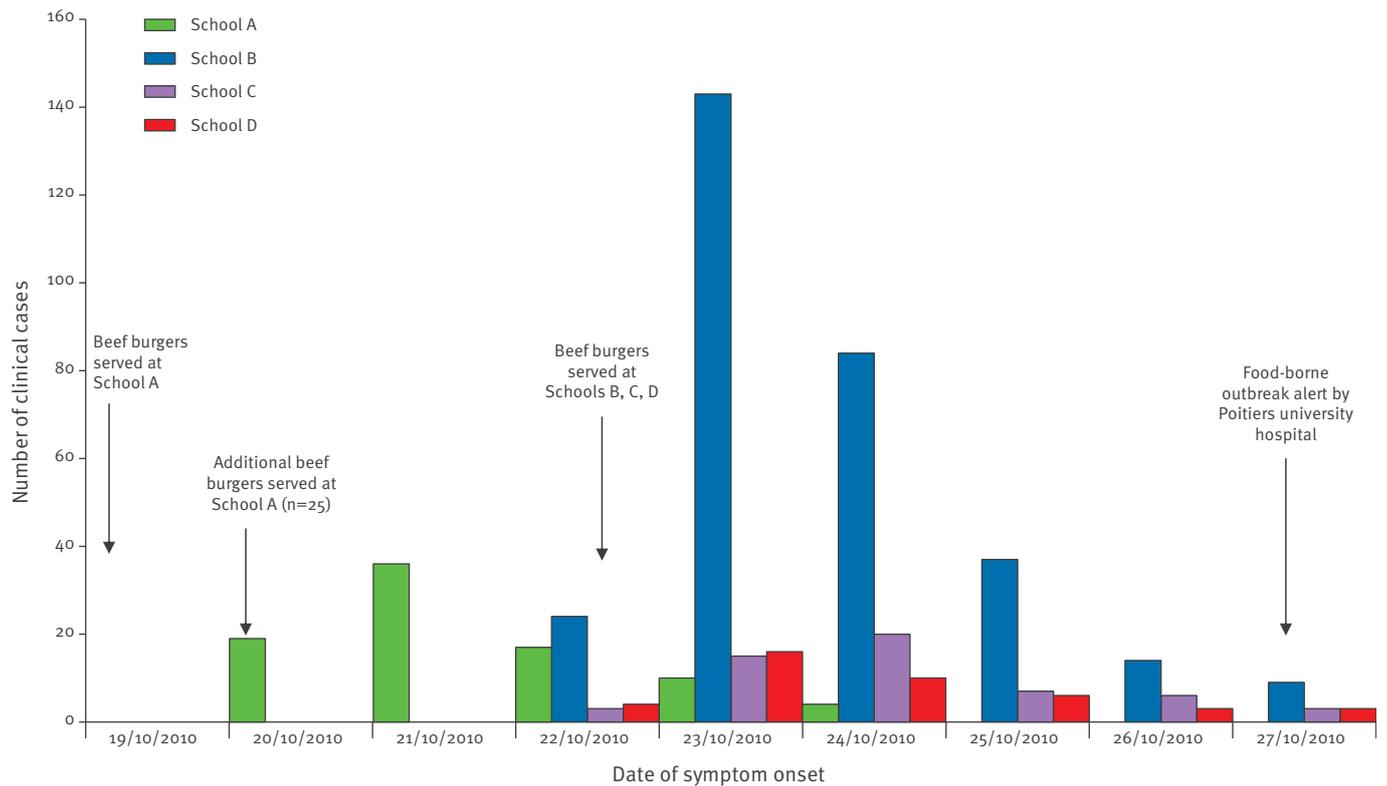
Among the 1,559 persons exposed, 554 were identified as clinical cases: 493 presented symptoms within five days after the lunch and 61 presented symptoms with an unknown time of onset within two weeks after the lunch. The global attack rate was 35.5% (554/1,559).

Attack rates were similar among persons of female and male sex (Table 3) (proportion test; p=0.46).

Attack rate was significantly higher (proportion test, p<0.001) among adolescents (<20 years-old) (534/1,474, 36%) compared to adults (10/65, 15%) although rates

FIGURE

Clinical cases by date of symptom onset and school, *Salmonella* outbreak in schools, Poitiers, France, October 2010 (n=493)



varied among schools (Table 3). The risk of developing symptoms of gastroenteritis after eating the incriminated lunch was 2.3 times greater for adolescents compared to adults (95% CI: 1.3–4.2).

Within the adolescent age group, the risk of developing symptoms of gastroenteritis decreased significantly with increasing age (chi-squared test for a linear trend=40.2, $p<0.001$).

Attack rates calculated per school are shown in Table 3. The highest attack rate was found for School B (46.8%). Attack rate was significantly lower for School C (17%) than for each of the other schools (proportion test, $p<0.001$). The risk of developing symptoms of gastroenteritis after eating lunch at one of the three Schools A, B and D was 1.78 times higher than for School C (95% CI: 1.43–2.21). The risk of developing symptoms of gastroenteritis after eating lunch at School B was 2.76 times higher than that for School C (95% CI: 2.22–3.44).

Among the 554 clinical cases, the three most frequently reported clinical symptoms were diarrhoea (490 cases, 88%), abdominal pain (451 cases, 81%) and fever (389 cases, 70%) (Table 4). Other reported symptoms included nausea (250 cases, 45%) and vomiting (179

cases, 32%). Table 4 describes the frequency of symptoms in cases per school.

More than half of cases (53 %) reported having consulted a doctor in a private practice or in a hospital setting. A total of 31 cases (6%) reported having been hospitalised for at least 24 hours (Table 4). Median hospital stay calculated for 27 of the 31 hospitalised cases was three days (95% CI: 1–4). A total of 41 cases (7%) declared having a stool culture positive for *Salmonella* (n=33) or having a ‘positive’ result (test not specified) (n=8).

The median incubation time calculated for the 296 cases who reported the time of onset of their symptoms in addition to the date of onset was 40 hours (95% CI: 27–56) (Table 4). The first case occurred one hour after lunch and the last case 127 hours after lunch. Median incubation was shortest (34 hours) for cases attending School B and longest (49 hours) for cases attending School A.

Among the 1,005 persons who had lunch the day the incriminated beef burger was served but who did not fit the case definition, 52 reported gastrointestinal symptoms beginning more than five days after the lunch (range: 6–34 days). Of these, 34 reported having

TABLE 3

Attack rate per age group, sex and school, *Salmonella* outbreak, Poitiers, France, October 2010 (n=554)

	Cases / exposed	Attack rate % (95% confidence interval)
Age group^a		
Adolescents (< 20 years-old)	534 / 1,474	36.2 (33,8–38,7)
Adults (≥20 years-old)	10 / 65	15.4 (7,6–26,5)
Sex^a		
Female	261 / 752	34.7 (31,3–38,2)
Male	287 / 786	36.5 (33,1–40,0)
School		
A ^b	95 / 268	35.4 (29,7–41,5)
B ^b	333 / 712	46.8 (43,0–50,5)
C ^b	76 / 449	17.0 (13,6–20,7)
D ^c	50 / 130	38.5 (30,1–47,4)
All schools	554 / 1,559	35.5 (33,1–38,0)

^a For persons with available information.

^b Junior high school (median age of students: 16 years).

^c Senior high school (median age of students: 12–13 years).

diarrhoea only and 18 reported fever with at least one digestive symptom.

Microbiological results

The two food isolates from frozen and from cooked beef burger sampled in the canteen of Schools A and B were monophasic *Salmonella* Typhimurium 4,5,12:i:- with resistance to ampicillin, streptomycin, sulphonamides and tetracycline (R(resistant)-type ASSuT), which is common to this strain [9]. Concentration of bacterial inoculum was 270 to 18,000 (colony-forming unit) CFU/g for the 23 enumerations carried out.

The NRC confirmed the presence of monophasic *Salmonella* Typhimurium 4,5,12:i:- (R-type ASSuT) in the 25 human isolates. Among these, 21 belonged to students who satisfied the case definition used in the study, three belonged to students who were ill but who did not satisfy the case definition used in the study (one with fever only and two with time of onset at nine and 10 days after the incriminated lunch) and one belonged to a student who did not participate in the questionnaire survey.

Food and human isolates exhibited a unique PFGE profile (XTYM-151) and a unique MLVA profile (3-14-6-NA-211).

Discussion and conclusion

A local school outbreak of monophasic *Salmonella* Typhimurium 4,5,12:i:- caused by imported food was identified through the national disease notification

system for food-borne illness. Investigation by the Food Control Unit quickly identified the cause of the outbreak as beef burgers and all batches of the same date than the incriminated batch were rapidly withdrawn. The impact of the consumption of the incriminated beef burgers was unknown at the time of outbreak notification as it occurred during school vacation. The retrospective epidemiological investigation conducted in the four affected schools of Poitiers enabled us to identify 554 clinical cases among the 1,559 study participants who ate at school on the days the incriminated beef burgers were served. Twenty-one cases who participated in the study were biologically confirmed by the NRC by serotyping.

This multi-school outbreak is one of the biggest food-borne outbreaks due to monophasic *Salmonella* Typhimurium 4,5,12:i:- described in France. A previously described large outbreak of *Salmonella enterica* serotype 4,[5],12:i:- in France involved 337 identified cases and occurred nationwide between 31 October and 18 December 2011 [10]. Another nationwide epidemic involving the same *Salmonella* strain occurred in France between 1 August and 9 October 2011 with 682 cases reported [10]. In these two outbreaks, the investigations indicated dried pork sausage as being the most likely source of the outbreaks [10]. The Poitiers school outbreak is the first large scale outbreak described in France of monophasic *Salmonella enterica* variants involving beef.

The overall attack rate of the Poitiers outbreak was higher than the average attack rate (7%) observed in *Salmonella* food-borne outbreaks that occurred in school canteens in 2006–2008 in France [1]. In the same period, no large-scale *Salmonella* outbreak was described in a school setting. The largest food outbreak described in schools in France occurred in 2008 and involved 1,137 identified cases with a global attack rate of 50% and was of viral origin [14].

Although the Poitiers outbreak occurred two months before the annual winter peak of viral gastroenteritis, we cannot exclude that some of the cases identified in the survey were due to other gastrointestinal disease. The high attack rate observed in this outbreak could be explained by a particularly high initial concentration of bacterial inoculum. A meta-analysis by Teunis et al. [15] on food-borne outbreak data showed median infective dose (ID₅₀) values ranging between 30 and 50 CFU/g for *Salmonella*.

The survey revealed an attack rate twice as high among adolescents as among adults. Moreover, the attack rate decreased with increasing age among adolescents. There are no obvious explanations for this finding other than the possibility that the younger students were served lunch at a different time than the others and thereby ate beef with a potentially different cooking time.

TABLE 4

Characteristics of clinical cases with symptoms of gastroenteritis, *Salmonella* outbreak, Poitiers, France, October 2010 (n=554)

	School A n(%) ^a	School B n(%) ^a	School C n(%) ^a	School D n(%) ^a	All schools n(%) ^a
Median incubation time in hours, IQR (number of cases) ^b	49, 43–69 (n=41)	34, 25–5 (n=204)	46, 35–68 (n=22)	39, 30–70 (n=29)	40, 27–56 (n=296)
Clinical symptoms	95 (100)	333 (100)	76 (100)	50 (100)	554 (100)
Nausea	56 (41)	164 (49)	30 (39)	17 (34)	250 (45)
Vomiting	34 (36)	119 (36)	12 (16)	14 (28)	179 (32)
Abdominal pain	74 (78)	285 (86)	52 (68)	40 (80)	451 (81)
Fever	72 (78)	254 (76)	40 (53)	23 (46)	389 (70)
Diarrhoea	82 (86)	295 (89)	65 (86)	48 (96)	490 (88)
Other symptoms	16 (17)	45 (14)	14 (18)	1 (2)	76 (14)
Treatment	95 (100)	333 (100)	76 (100)	50 (100)	554 (100)
Doctor consultation	48 (51)	201 (62)	18 (76)	19 (62)	286 (53)
Hospitalisation for at least 24 hours	6 (6)	22 (7)	2 (3)	1 (2)	31 (6)
Stool culture	16 (17)	51 (17)	7 (9)	4 (8)	78 (15)

IQR: interquartile range.

^a Unless otherwise specified.

^b For cases who reported the time of onset of their symptoms in addition to the date.

The outbreak showed signs of severity with about half of the cases who sought medical care in a private practice or an emergency service, of which 31 of 554 (6%) were hospitalised for more than 24 hours.

Incubation for *Salmonella* infection is known to range from six to 72 hours with longer incubations (up to 16 days) documented [16]. Two students with a positive stool culture and an incubation of nine and 10 days could be secondary cases contaminated by person-to-person spread. We observed that median incubation was shortest in the school with the highest attack rate (School B). Such negative correlation between attack rate and incubation has been documented in previous outbreaks and retrospective analysis of human outbreaks [17]. This highlights the possibility that the infective dose was greater in beef burgers served in School B than in other schools, in particular School C. Moreover, hospitalisation rate used as a proxy measure for disease severity in this context was 2.6 times greater among cases attending School B than among those attending School C. However, the possibility that frozen beef burgers were contaminated at different concentrations is unlikely because beef burgers supplied to schools were all from the same batch. One hypothesis for different attack rates observed between schools relates to different cooking practices.

Although the number of cases identified by the investigation is probably close to the real number of cases of salmonellosis due to ingestion of contaminated beef burgers, the number could be underestimated because of non exhaustive study participation (response rate

78%), because of our assumption that all those who ate at the school consumed the beef, and because of errors in reporting disease onset for persons with clinical symptoms. Inversely, important local media attention before the conduct of the investigation could have induced a high participation rate of the ill student group and an over declaration of symptoms due to psychogenic-like effect. The two-week delay between the first cases and the questionnaire survey (due to school vacation) could have led to recall bias and errors, especially in date reporting. The risk of error for exposure was minimised by asking students about the date they ate lunch at school instead of asking them the specific food items they had eaten.

At the country's departmental level, two other localised outbreaks of monophasic *Salmonella* Typhimurium 4,5,12:i:- occurred in October 2010, one in a retirement institution and another in a recreation centre associated with the consumption of beef burgers from the incriminated batch (18 cases, unpublished data). Interviewing at each of the eighteen other institutions in the department that received frozen beef burgers with the same production date as the incriminated batch revealed that none was aware of cases of diarrhoea within their institution (as of 9 November 2010). Although distribution of potentially contaminated frozen beef burgers was widespread in France, no increase in food-borne outbreaks was detected through disease notification surveillance and no local increase in the serotype 4,5,12:i:- was detected by the NRC (as of 9 November 2010).

The international dimension of this outbreak in France is demonstrated by the fact that the beef was produced in an establishment in another EU Member State. The Rapid Alert System for Food and Feed (RASFF) was used to inform authorities of the manufacturer country and other EU countries likely to receive the products. Only a French distributor received all the batches from this Member State manufacturer and sold them. Furthermore, no food-borne outbreaks caused by the monophasic *Salmonella* Typhimurium 4,5,12:i:- were reported in Europe at the time of the outbreak in Poitiers.

Monophasic *Salmonella* Typhimurium 4,5,12:i:- was associated with a severe outbreak, the largest *Salmonella* food-borne outbreak described in a school setting in France in recent years. Quick identification and withdrawal of incriminated food batch and respect of safe cooking practices for beef burgers were likely crucial to limit extension of outbreak. Informing other European countries was necessary as the incriminated beef was an imported food product.

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