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A community-wide outbreak of *Salmonella enterica* serotype Typhimurium infection associated with eating a raw milk soft cheese in France

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SUMMARY

In 1997, a community-wide outbreak of *Salmonella enterica* serotype Typhimurium (*S. typhimurium*) infection occurred in France. The investigation included case searching and a case-control study. A case was defined as a resident of the Jura district with fever or diarrhoea between 12 May and 8 July 1997, from whom *S. typhimurium* was isolated in stool or blood. One hundred and thirteen cases were identified. Thirty-three (83%) of 40 cases but only 23 (55%) of 42 community controls, matched for age and area of residence, reported eating Morbier cheese (Odds ratio: 6·5; 95% Confidence Interval: 1·4–28·8). Morbier cheese samples taken from the refrigerators of two case-patients and one symptom-free neighbour cultured positive for *S. typhimurium* of the same phage type as the human isolates. The analysis of distribution channels incriminated one batch from a single processing plant. These findings show that an unpasteurized soft cheese is an effective vehicle of *S. typhimurium* transmission.

INTRODUCTION

Non-typhoidal salmonella are among the main causes of food-borne illness in the 'western world' [1]. In France, for many years, *Salmonella enterica* serotype Typhimurium (*S. typhimurium*) has been the commonest serotype isolated from human cases, although from 1990–4 it was overtaken by *Salmonella enterica* serotype Enteritidis [2]. *S. typhimurium* is the commonest salmonella infection in cattle [3] and is the most frequent serotype among salmonella isolates from beef products as well as pork and pork meat products [4]. Several outbreaks of *S. typhimurium* food poisoning associated with eating beef have been reported [5, 6]. Pork and pork meat products have been implicated in outbreaks in England [7], Wales [8] and Italy (9) as well as in a case-control study in Britain [10]. The only community-wide outbreak of *S. typhimurium* infection reported in France was found to be associated with eating roasted chicken from a local supermarket [11].

In late May 1997, staff of the District Health Department of the Jura district were informed by the physicians of a local hospital and a medical laboratory of 20 cases of *S. typhimurium* infection that had occurred since 20 May. The patients resided in different communities in the south of the Jura and had

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not participated in a common meal or social event. Following these reports, the District Medical Officer contacted the main hospitals and medical laboratories in the district requesting that they report cases of *S. typhimurium* infection. In collaboration with district officers of the Veterinary Services of the Ministry of Agriculture and of the Consumers Affairs Directorate of the Ministry of Finance, an outbreak investigation committee was established. Several patients were interviewed on food consumption in the 3 days prior to their illness using a questionnaire mainly targeted at meat and poultry products. Food samples were taken from shops where patients had purchased these products. The initial results of these investigations showed that several cases had eaten barbecued chicken. However, the origins of the chicken varied widely and there was no common supplier. Cultures of food samples were all negative for *S. typhimurium*, with the exception of a pork cutlet, a food eaten by only few of the cases. Since the preliminary investigations remained inconclusive and faced with an increasing number of cases, the District Medical Officer requested the assistance of the Réseau National de Santé Publique in the investigation. This paper describes the methods and results of the collaborative investigation carried out.

METHODS

Epidemiological investigation

A confirmed case was defined as *S. typhimurium* isolated from a faecal or blood specimen in a resident of the Jura district or neighbouring communities, after 12 May 1997. A probable case was defined as a resident of the Jura district or neighbouring area, with fever (> 38 °C) or diarrhoea (at least three loose stools daily), occurring in the week preceding or following the onset of symptoms of a confirmed case in the same household. Cases were identified by contacting, at least weekly, all public and private medical laboratories in the district. To generate an hypothesis 17 cases were interviewed by telephone using a long semi-structured questionnaire designed to enquire about meat and meat products, poultry, egg and dairy products, and other foods including regional products, consumed in the 2 weeks prior to the onset of symptoms. This preliminary investigation revealed a high proportion (14/17) of case-patients having eaten Morbier, a regionally produced soft cheese made from raw cows' milk, and a high proportion (13/17) having eaten Comté, a regionally produced cows' milk hard cheese. To test the hypothesis that one of these cheeses was the vehicle of infection in this outbreak, we conducted a case control study. Patients were included as cases if they had had diarrhoea (at least three loose stools daily) or fever (> 38 °C), and if they had been the first case that had occurred in the household. Fourteen of the 17 case-patients interviewed in the preliminary investigation were included in the case-control study, but separate analyses were undertaken both with and without these cases. One control, identified by the case's general practitioner, was matched to each case on place of residence (within 30 km from the case) and age group (< 1, 1–5, 6–14, 15–64, $\leqslant 65$ years). Controls were excluded if they reported having had a diarrhoeal illness since 12 May 1997 or a history of travel out of the district in the period covered by the interview. Cases and controls were interviewed by telephone by the same interviewer for each pair, using a standardized questionnaire mainly targeted at regional dairy products as well as meat and poultry products. Subjects were also questioned regarding recent travel and exposure to a case of diarrhoea in the household. Cases and controls were interviewed 1–7 weeks after onset of symptoms of the case. Therefore, we asked for food consumption in the 2 weeks prior to illness of the case rather than during the exact 3 days before illness. We planned to include 54 cases and controls in order to have a power of 80% to detect an increased risk (estimated OR of 4) associated with the consumption of a regional cheese (consumption within the general population assumed to be 60%) significant at the 5% level. *S. typhimurium* isolation rates were calculated using data from the 1990 national census as denominators. Single variable analysis was carried out using Epi Info version 6.02. Matched Odds Ratios (OR) were calculated using Mantel Haenszel 95% confidence limits.

Environmental investigation

District officers of the Veterinary Services analysed the supply channels of the retail stores of the incriminated cheese and identified a common processing plant, which they inspected in late June. Hygiene procedures in cheese production and storage, microbiological monitoring of milk and cheeses, and illness in cattle supplying milk to the plant were reviewed. In addition, workers in the plant were questioned regarding diarrhoeal illness in the period of production of a batch which was incriminated

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during the investigation and stool specimens were taken from those present in the month of July.

Microbiological investigations

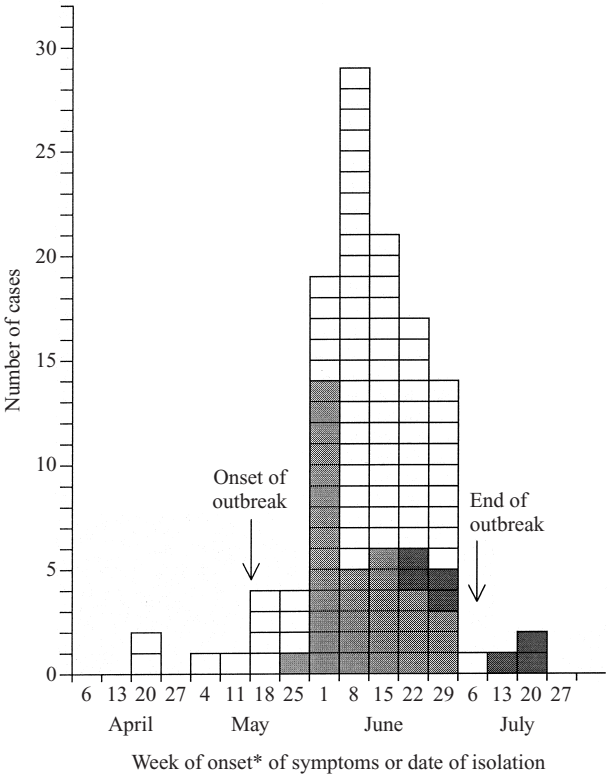
Food specimens taken from the incriminated and other foods in patients refrigerators, at the retail stores and in the incriminated processing plant, were examined for salmonella. *S. typhimurium* isolates (human and food) were confirmed and tested for antimicrobial susceptibility by the National Reference Centre for *Salmonella* and *Shigella* and phage typed by the National Reference Centre for Enteric Molecular Typing using the methods described by Callow [12] and Anderson [13].

RESULTS

Epidemiological investigation

From 12 May to 4 July 1997, a total of 109 confirmed and 4 probable case-patients were identified. The outbreak began during the third week of May and continued till the first week of July (Fig. 1). Of the 55 case-patients for whom the information was available. 11 (20%) were hospitalized. No deaths associated with this outbreak were identified. The isolation rate was 439 per million population and was highest in children aged 1–5 years (Table 1). The sex ratio was 1. Predominant signs and symptoms among 41 case-patients for whom this information was available included diarrhoea (98%), fever (73%), abdominal pain (83%), nausea (44%) and vomiting (34%).

By 4 July, food histories were completed for 42 case-patients and their controls. Although the sample size of 54 pairs had not been reached, it was decided not to continue the interviews out of fear that the information that a regional cheese was suspected began to spread as a result of food samples taken from case-patients refrigerators as well as from shops. The 42 cases did not differ from the total series of identified case-patients in sex ($P = 0{\cdot}8$), in their distribution over age groups ($P = 0{\cdot}3$) and in the proportion of hospitalized case-patients ($P = 0{\cdot}5$). When cases from the preliminary inquiry were excluded from the analysis, having eaten Morbier cheese was found to be associated with illness (matched OR 4·5; 95% confidence interval 1·0–21·9 (Table 2). This association became stronger when all cases were included in the analysis. Of the 40 case-patients who could give a definite answer, 33 (83%) reported having eaten Morbier cheese compared with 23 (55%) of the 42 controls (matched OR 6·5; 95% confidence interval 1·4–28·8) (Table 2). The consumption of other types of cheese or meat products was not associated with an increased risk of illness (Table 2).



* The date indicates the last day of the week.

Fig. 1. Number of confirmed cases of *S. typhimurium* infection by week of onset of symptoms or (if onset unknown) of isolation and by phage type of isolate, Jura, France, May–July 1997. □, Case, no phage type of isolate available; ▨, Case, isolate phage type 'DT12 atypical'; ■, Case, isolate phage type other than 'DT12 atypical'.

Table 1. *Confirmed cases of* S. typhimurium *infection by age group, Jura, May–July 1997*

Age group (years)	Number (%) of cases	Population	Isolation rate per 100000 population
< 1	2 (1·8)	3200	63
1–5	36 (33·0)	16000	225
6–14	22 (20·2)	30300	73
15–64	29 (26·6)	159500	18
⩾ 65	9 (8·3)	39100	23
Unknown	11 (10·1)	—	—
Total	109 (100)	248100	44

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Table 2. *Cases of* S. typhimurium *infection and controls by food consumption, Jura, France, May–July 1997*

Foods	Cases		Controls			
	Have eaten *n* (%)	Total†	Have eaten *n* (%)	Total†	Matched OR	95% CI
Mean and poultry						
Pork	23 (59)	39	29 (76)	38	0·5	0·2–1·5
Sausage	24 (57)	42	28 (67)	42	0·7	0·3–1·6
Minced beef	20 (48)	42	18 (43)	42	1·2	0·5–3·3
Ready cooked chicken	15 (36)	42	10 (24)	42	1·7	0·7–4·4
Non regional cheese						
Camembert	21 (54)	39	30 (71)	42	0·4	0·2–1·1
Regional cheese						
Comté	36 (86)	42	37 (88)	42	0·8	0·3–2·7
Morbier (*all cases*)	33 (83)	40	23 (55)	42	6·5	1·4–28·8
Morbier (*subset of cases**)	22 (79)	28	15 (54)	28	4·5	1·0–20·8

* Cases from preliminary inquiry excluded from the analysis.
† Total of cases and controls that could give a definite answer to the question.

Environmental and microbiological investigations

Food specimens were collected from left over cheeses (4 Morbiers, 3 Comtés, 1 Bleu de Gex) and butter in the refrigerators of 5 case-patients as well as from a symptom-free neighbour of a case-patient who had purchased Morbier in the same period and at the same place as the case-patient. Three of the Morbier specimens, including the Morbier of the symptom-free neighbour, and none of the other food specimens, cultured positive for *S. typhimurium*. Morbier is a soft cheese made from raw cows' milk. There are 20 producers in the Jura district. Cheeses are stored for approximately 45 days for maturation. Usually there is no 'use by' date but, in general, shops sell the cheese as an unlabelled product at the delicatessen counter within a month of it being put on the market.

Fourteen of the 22 retail shops mentioned by 24 case-patients who were able to recall one or several places of purchase of Morbier, were found to be supplied by two wholesalers who had purchased Morbier cheese on the same day (6 May) at one processing plant (plant A). Of the 24 case patients, 18 had consumed Morbier that could be linked to this plant, 2 case patients had consumed Morbier from a different producer, and for 4 cases the supplier of their retail store could not be identified with certainty. Also, one of the Morbier cheeses that was culture positive for *S. typhimurium* was traced back as a cheese produced by the incriminated producer. Due to the fact that most shops sell Morbier cheese from different producers at the same time, the producer of the two other *S. typhimurium* positive Morbier cheeses could not be identified with certainty although the incriminated producer figured among the suppliers of the retail shops in the period that the case-patients had purchased their cheese. These findings allowed us to identify one specific batch of Morbier cheese, batch number 67, produced by plant A on 8 March and sold to the wholesalers on 6 May 1997.

None of the specimens of Morbier cheese taken at places of purchase of cases cultured positive for *S. typhimurium*. It was later shown that none of the specimens had come from the incriminated batch of Morbier.

The environmental investigation of the processing plant did not reveal any deficiencies in hygienic procedures. Approximately every other day, specimens were taken from the production of that day and examined for salmonella. No specimen had been taken from the incriminated batch number 67, but earlier and later batches (62, 65, 66, 70, 71, 72, 76) as well as a different type of soft cheese produced on the same day as the incriminated batch, cultured negative for salmonella. Among the workers there had been no absenteeism for reason of diarrhoea in the month of production of the implicated batch. Seventeen of the 27 workers that had a stool specimen taken in July, were all negative for salmonella. No illness had been reported among the cattle supplying milk to the plant, in the 3 months prior to production of the incriminated batch. In July, milk samples of the 100 farms supplying the plant were examined for salmonella and cultured negative.

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There was no recall of the incriminated batch because it was considered certain that by the 9 July, the day that the batch was identified as the likely vehicle of infection, all the batch would have been exhausted. This was consistent with the finding that during the investigation in the retail shops which took place between 16 June and 8 July, no Morbier of the incriminated batch had been found to be still on sale.

The incriminated batch consisted of 600 Morbier cheeses of 20 kg each. Two wholesalers had bought 300 cheeses and supplied them to retail stores in the south and the north of the Jura district corresponding to the geographic distribution of the cases, as well as to retail stores in the capital of the neighbouring district Côte d'Or. No active case-finding was carried out in this district. However, the National Reference Centre, which receives isolates of salmonella on a regular basis from correspondent laboratories, noted an increase in isolates of *S. typhimurium* sent by microbiologists in the Côte d'Or district, with 21 isolates received between 12 May and the 11 July compared to only 8 isolates received in the period May, June, July in 1996. In addition, over the same period, the university hospital in Côte d'Or reported eight cases of *S. typhimurium* infection. The rest of the incriminated batch had been distributed in seven districts. The National Reference Centre did not note an increase in the number of isolates from any of these districts.

The *S. typhimurium* strains of 37 case-patients isolated between 12 May and 4 July and of 3 patients with onset of disease after 4 July were sent to the National Reference Center. Among the first 37 isolates, 33 were shown to belong to the phage type 'DT12 atypical', which differs from the international definitive type 104 (DT104) by one phage reaction (Fig. 1). None of the *S. typhimurium* isolates of the three patients with onset of disease in the second and third week of July belonged to the 'DT12 atypical' phage type. The strains isolated from the three Morbier cheeses were shown to belong to the same phage type as the isolates of the epidemic cases. Antimicrobial sensitivity testing was completed on the 3 food isolates and on 8 isolates drawn randomly from the human DT12 atypical isolates. All 11 isolates were resistant to ampicillin, chloramphenicol, streptomycin, sulfamethoxazole and tetracycline (ACSSuT).

DISCUSSION

The results of the investigation indicate that this outbreak of salmonellosis was caused by a raw milk cheese, Morbier, from a single processing plant and most likely belonging to one batch. Evidence comes from the results of the case-control study, the isolation of *S. typhimurium* from Morbier cheese belonging to the same phage-type and with the same antimicrobial resistance pattern as the human isolates, and the fact that the outbreak started within a few days of the batch being put on the market and that no new cases occurred after the implicated batch was exhausted.

Recall bias may have been introduced by the fact that case-patients, seeking an explanation for their illness, would be more likely to remember their food consumption than controls. However, this would apply to other food items as well. Also, data collection was stopped before the information that a regional cheese was the suspected vehicle of infection started to spread. Because interviewers were not blinded to case or control status, bias could have been introduced if case-patients were questioned differently than were controls. This possibility was minimized by the use of a highly structured questionnaire. Therefore, it is unlikely that the introduction of these types of bias alone would explain the association with Morbier cheese. Since the food isolates of *S. typhimurium* all came from left over cheese it could be argued that the culture positive Morbier cheese had been contaminated by the case-patients during handling of the cheese. However, the fact that other types of cheese in the case-patients' refrigerators cultured negative, and the isolation of *S. typhimurium* from Morbier cheese purchased by a symptom-free person in the same period and at the same place as a case-patient, strongly argue against this hypothesis.

The mechanism by which the batch of cheese was contaminated at the incriminated plant remains unclear. The absence of *S. typhimurium* in batches produced before and after the incriminated batch, and the fact that no new cases were detected after the incriminated batch was exhausted, suggest a point source of contamination. This source could have been either environmental, animal or human. Contamination of the milk may have taken place during milking, storage or transport. Although no employees had reported gastro-intestinal illness in the period of production of the implicated batch, contamination during the production process by a member of personnel carrying *S. typhimurium* cannot be excluded. The frequency of bacteriologic quality monitoring as practised by the incriminated plant was insufficient to enable the detection of a transient contamination. Also, bacteriological control of

samples of the end product may be unsuccessful to detect a contaminated product if only a small proportion of the product is contaminated or if the level of contamination is very low. As demonstrated in other studies, large outbreaks can result even if a small percentage of the implicated food products are contaminated, and extremely low levels of salmonella (< 1 cell per 100 g) are present in the food [14, 15]. Therefore, in addition to bacteriological quality monitoring of the end product, hazard analysis critical control point procedures should be implemented for unpasteurized as well as pasteurized products.

This outbreak was characterized by a high attack rate, with a substantially higher isolation rate of *S. typhimurium* than reported in other community-wide outbreaks of salmonellosis (16). Since the epidemic strain is the most frequent phage type of *S. typhimurium* in France, some of the cases may have been sporadic cases. However, in the weeks following the outbreak, when active laboratory surveillance was maintained, no more than two isolates per week were reported, suggesting that sporadic cases made up only a minor proportion of the total cases identified during the outbreak. Therefore, considering that, in France, as few as 6% of diarrhoea patients who consult their general practitioner are estimated to have a stool culture [17], and that many patients do not see a doctor at all, the true number of epidemic cases may well have been several thousand.

Raw milk cheese has been implicated as the vehicle of infection in several recent outbreaks of salmonellosis. *Salmonella* Dublin was the causative organism of an outbreak in 1989 in England and Wales [18] as well as an outbreak in France in 1995 [19], both linked to the consumption of an unpasteurized soft cheese. In 1993, a nation-wide outbreak of *Salmonella* Paratyphi B infection in France was traced to a cheese made from unpasteurized goats' milk [16]. Outbreaks of salmonellosis with pasteurized cheese as vehicle of infection have been described as well. In 1980, an outbreak of *Salmonella* Heidelberg infections in the USA was caused by contaminated cheddar cheese [15]. This outbreak was shown to have arisen from deficiencies in pasteurization procedures for milk used in the manufacture of cheese. However, in 1989, investigation of an outbreak of *Salmonella* Javiana and *Salmonella* Oranienburg infections linked to the consumption of contaminated shredded cheese and mozzarella cheese identified no deficiencies in pasteurization [14].

The above mentioned outbreaks have shown that cheese is an efficient vehicle for salmonella. Consequently, people most susceptible to salmonella infection (infants, pregnant women, elderly and immunocompromised patients) should be advised to avoid raw milk cheeses, which are at highest risk of being contaminated. As a prerequisite to this recommendation, correct labelling of all dairy products, including those sold at the delicatessen counter, indicating if the product is made from raw milk, must be enforced.

To our knowledge, this is the second reported outbreak of *S. typhimurium* infection linked to the consumption of a raw milk soft cheese, an earlier outbreak being reported in Switzerland in 1985 [20]. It is surprising that so few outbreaks of *S. typhimurium* infection linked to cheese have been reported considering that, in France, *S. typhimurium* is the most frequent serotype isolated from dairy products, accounting for > 35% of salmonella isolates [21]. Nevertheless, most of the reported outbreaks were caused by relatively rare serotypes. A possible explanation could be that recognition of a community outbreak of salmonellosis and establishing its source can be difficult especially if the disease is caused by a common organism such as *S. typhimurium*, and if the vehicle of infection is a common food item. In this case, recognition of the outbreak and the vehicle of infection was aided by the geographical clustering of cases and the high attack rates, but it is possible that outbreaks in which cases are geographically scattered go undetected. Therefore, failure to detect outbreaks should not alone be used to presume safety.

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